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Laboratory Manual

by George Zahrobsky



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Foreword

Welcome to the biology laboratory. Here, you will work with the ideas and principles of the science of biology. In addition, you will learn many techniques and skills used by biologists. These include devising and performing experiments, gathering data by making observations and measurements, and analyzing the information you obtain.

Each laboratory investigation in this manual is organized into five sections. The **Purpose** states the goal of the investigation in one or two short phrases. The equipment and other items used in the investigation are listed under **Materials**. The **Introduction** provides the background information necessary to understand and successfully perform the investigation. The body of the investigation—the actual activities you will do—is described under **Procedure**. The investigation ends with the **Analysis** section, in which you are asked questions that sum up your laboratory experience. In addition to these five parts, many investigations end with a section called **Follow-up**. This suggests additional activities related to the investigation. Be sure to check with your teacher before performing the follow-up activities.

To be sure that a science laboratory is a safe place, precautions must be taken in dealing with chemical, biological, and physical materials. Listen carefully to your teacher's instructions at the beginning of each laboratory session regarding potential hazards in

the procedures and materials. Also, pay close attention to the notes in the margins of the investigations cautioning you about potential dangers. Learn where safety equipment, such as the fire extinguisher and eye wash station, is located and how to operate it. Never work alone or without supervision in the laboratory.

Accidents happen when you least expect them. If there were a gaping hole in the laboratory floor, probably no one would fall into it. Little, subtle things are more likely to cause accidents. Placing an acid bottle close to the edge of a lab table or putting a petri dish of bacteria on a stool for “just a minute” is asking for trouble. Spilled acid can be harmful; a dropped petri dish can ruin an experiment.

On the inside back cover of this manual are guidelines for safe procedures and first aid instructions for emergencies. Make a point of reading this information carefully before your first investigation and refer to it often during the laboratory course. Ask your teacher for further explanations if necessary. Remember to perform *only* those activities specified and approved by your instructor.

Working in the laboratory can be interesting and fun. Your success will depend on the amount of effort you expend. Always use caution, follow your teacher's instructions, and do your best work. Who knows, you might emerge from the laboratory ready for a career in science. Good luck!

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1 Data Gathering

PURPOSE

To learn methods of gathering data that are useful in the study of biology.

MATERIALS

balance	ruler
beaker	thermometer
10 mL graduated cylinder	boiling water

INTRODUCTION

When you work in a biology laboratory, you must often gather information. This information is usually called data. You will gather data while doing experiments and while observing things.

A vital part of data-gathering is measurement. Almost everyone has used measuring tools to gather data. When you are proficient in the use of these tools, your measuring—and therefore your data gathering—will be accurate.

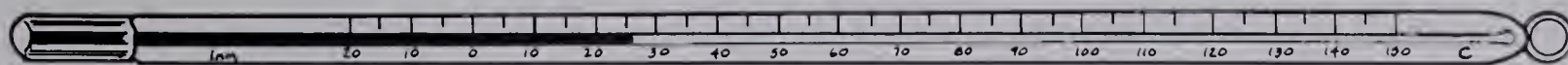
In this lab you will use four measuring tools: a thermometer, a balance, a graduated cylinder, and a ruler.

PROCEDURE

A. Using the Thermometer

The thermometer is an instrument used to measure temperature. In the metric system, the unit of measure used with the thermometer is the degree Celsius ($^{\circ}\text{C}$).

Take Care: The thermometer is a precise, delicate instrument and must be treated with care. Never use the thermometer as a stirring rod—the bulb may break.



The thermometer is a piece of glass tubing filled with either mercury or a colored liquid—usually alcohol. The tubing is marked off, or calibrated, in regular intervals. The bulb on the end is paper thin so that heat and cold can be transferred to the liquid inside as quickly and efficiently as possible. Heat causes the liquid to expand and rise in the tube, and cold causes the liquid to contract and fall. When the liquid stops moving, the calibration at the point where it stops tells you the temperature of what you are measuring.

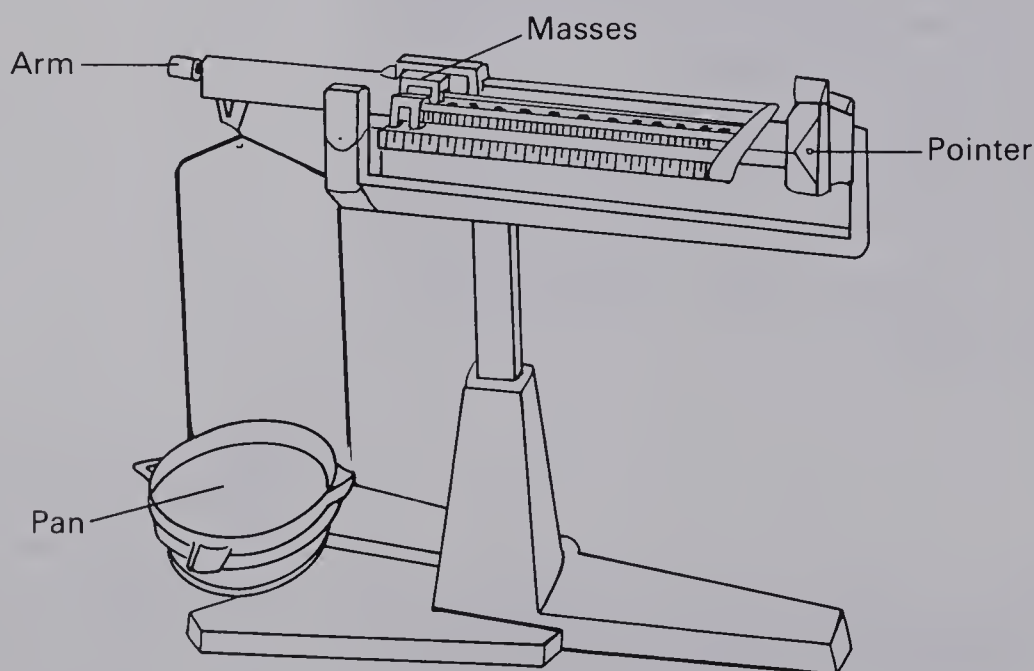
Determine the temperature of boiling water. Hold the thermometer in a beaker of boiling water so that the bulb is immersed but does not touch the bottom or sides of the beaker. Keep the bulb in the water at least one minute, or until there is no further temperature change. Do not let any ideas you might have about the temperature of boiling water influence your reading.

1. According to the thermometer, what is the temperature of boiling water?

Put your reading on the chalkboard. Determine the range of readings from low to high in your class.

B. Using the Balance

The balance is used to measure the mass of something. The metric system unit of measure used with the balance is the gram (g). The gram is a unit of mass.



Check the balance to be sure that it is zeroed. The weights, or masses, should be at zero. The arm must be released and able to swing freely. Nothing should obstruct the movement of the pan. Is the balance zeroed? If not, *do not attempt to adjust the balance yourself*. Ask your teacher for help.

Balances are designed to be used with certain pans. Pans on balances are not interchangeable. Often, the serial number of the balance or a school identification mark is stamped on the underside of the pan. Check the number of the pan against the number on the balance to make certain they are the same.

If the pan on the balance is dirty, wipe it clean with a damp paper towel and dry it before use. Do not place the material you are measuring directly onto the pan. Place it either on a piece of paper or in a beaker, and set that on the balance pan.

You will use the balance to measure 9 g of water in a beaker. First you must determine the mass of the empty beaker. Place the beaker on the pan. Move the weights until the pointer begins to drop. Start with the heaviest weight that will move the pointer but not cause it to drop to the bottom immediately. Then, move the lighter weights until the pointer points at the center position. Check the positions of the weights to determine the mass of the beaker. Then add 9 g to this figure and set the weights on the balance to the total you calculated.

Slowly pour cold tapwater into the beaker until the balance swings to the center position.

2. What is the mass of the beaker? _____ g

3. What is the mass of the water you added? _____ g

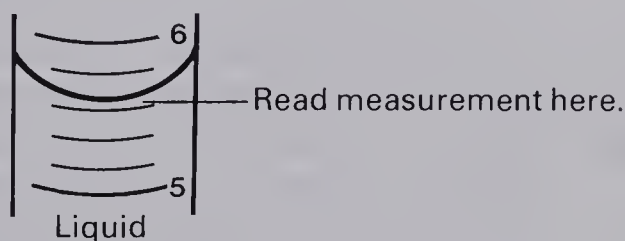
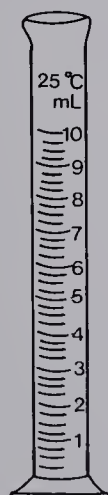
4. What is the total mass of the beaker and water? _____ g

Ask your teacher or a classmate to check your data and initial here:

Save the water in the beaker for the observation in part C.

C. Using the Graduated Cylinder

The graduated cylinder is used to measure the volume of a liquid. The metric unit for volume is the millilitre (mL). *Milli* means thousandth: there are 1000 mL in a litre.



Meniscus (curvature exaggerated)

When you pour a liquid into any container, it is attracted to the side walls of the container. By looking closely, you can see that the surface of the liquid in the cylinder is not flat. It curves up against the side walls. This curved surface is called the meniscus. To make the correct measurement, read the position of the *bottom* of the meniscus.

Pour the 9 g of water you saved when doing part B into the graduated cylinder.

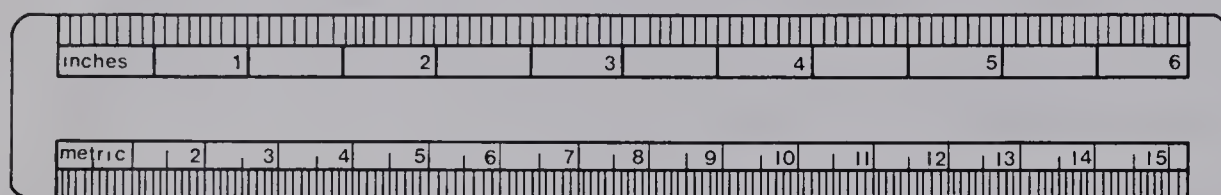
5. What is the volume of 9 g of water? _____ mL

Ask your teacher or a classmate to confirm the reading of the volume of the water in the graduated cylinder here:

_____ mL

D. Using the Ruler

The ruler is a simple but highly useful instrument of measure. It is a straightedge graduated in linear units. The smallest unit on the metric ruler is the millimetre (mm). The length of 1 mm is roughly equal to the thickness of a dime. The centimetre (cm) is ten millimetres. Usually the numbers printed on a metric ruler represent centimetres.



6. Measure the length of this page in cm and in mm. If the page falls between marks on your ruler, record the length to the nearest mark. Record your data here.

ANALYSIS

7. Why should a thermometer not be used as a stirring rod?

8. The boiling point of water at sea level is 100°C . Give two reasons why the thermometer might not register 100°C when placed in boiling water.

9. What is a meniscus?

10. Fifteen centimetres can be expressed as how many mm?

2 Graphing

PURPOSE

To learn methods of presenting data that are useful in the study of biology.

MATERIALS

pencil

ruler

INTRODUCTION

There are different kinds of situations in which you collect data. For example, you might want to observe the temperature changes in an oak and hickory forest in Tennessee over the course of one year. You then collect the necessary data. Once a day you would measure the temperature in the forest, then average the daily temperatures for each month of the year.

Another situation in which you would gather data is an experiment. You might want to find out how much fertilizer will produce the most growth of a plant. You would run an experiment, applying fertilizer in various concentrations to plants. Then you collect the data: how much each plant grows.

Still another data-collection situation is finding the size distribution of something. In a group of 26 seeds, for example, what is the length of each seed? How many seeds are the same length? What is the most common length? In your data-gathering you would measure each seed.

Once you have collected data, you must analyze it to find out what it means. Displaying the data is a great help in making an analysis. One method of display is to organize the data into a table (see the examples). Tables, however, are of limited use.

A more informative method of displaying data is to graph it. Graphs represent data visually. Often, important facts about the data can more easily be seen in a graph than in a table.

There are many kinds of graphs, and two are especially useful in displaying data that a biologist collects. These are line graphs and histograms.

PROCEDURE

A. Making a Line Graph

Data From Experiments This kind of data is often displayed on a line graph. A line graph would be used to show the effect of fertilizer concentration on plant growth.

To graph the data in Table 1, you must first determine the variables. The independent variable is always controlled by the experimenter. In this case, the experimenter decided which concentrations of fertilizer to apply to the plants. Thus, the percent fertilizer concentration is the independent variable.

The dependent variable is the factor that is being measured. Each value of the dependent variable *depends* on a corresponding value of the independent variable. In this case, the dependent variable is plant growth. How much the plant grows depends on the concentration of the fertilizer. The dependent variable represents the data collected.

On the line graph, the independent variable is always marked along the horizontal X-axis. The dependent variable is always marked along the vertical Y-axis.

The scale for each axis can be marked in several possible ways. You can start at any number, not necessarily zero, and you can have any number of spaces represent one unit of measurement. For example, you might decide that one space on the Y-axis represents 1 mm of growth, as in Graph 1. The scale you choose must be uniform for the entire axis. If one space represents 1 mm of growth, three spaces must represent 3 mm of growth.

The scales do not have to be set up the same way on both axes. However, the scales must accommodate the ranges of the two variables. The range of fertilizer concentration is from 1 percent to 9 percent. The range of plant growth is from 2 mm to 8 mm. The range of the scale should be just a little larger than the range of the data so that the data fit on the graph.

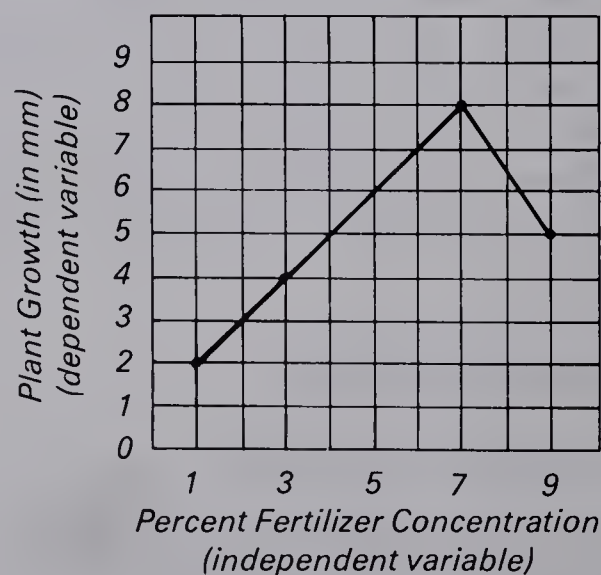
There is not one right scale. The goal is to fit all the data on the graph and to use most of the graphing space.

1. Study Graph 1, "Effect of Fertilizer on Plant Growth." How much growth would you predict at 4 percent fertilizer concentration?

Table 1. Effect of Fertilizer on Plant Growth

<i>Fertilizer concentration (percent)</i>	<i>Plant growth (in mm)</i>
1	2
3	4
5	6
7	8
9	5

Graph 1. Effect of Fertilizer on Plant Growth



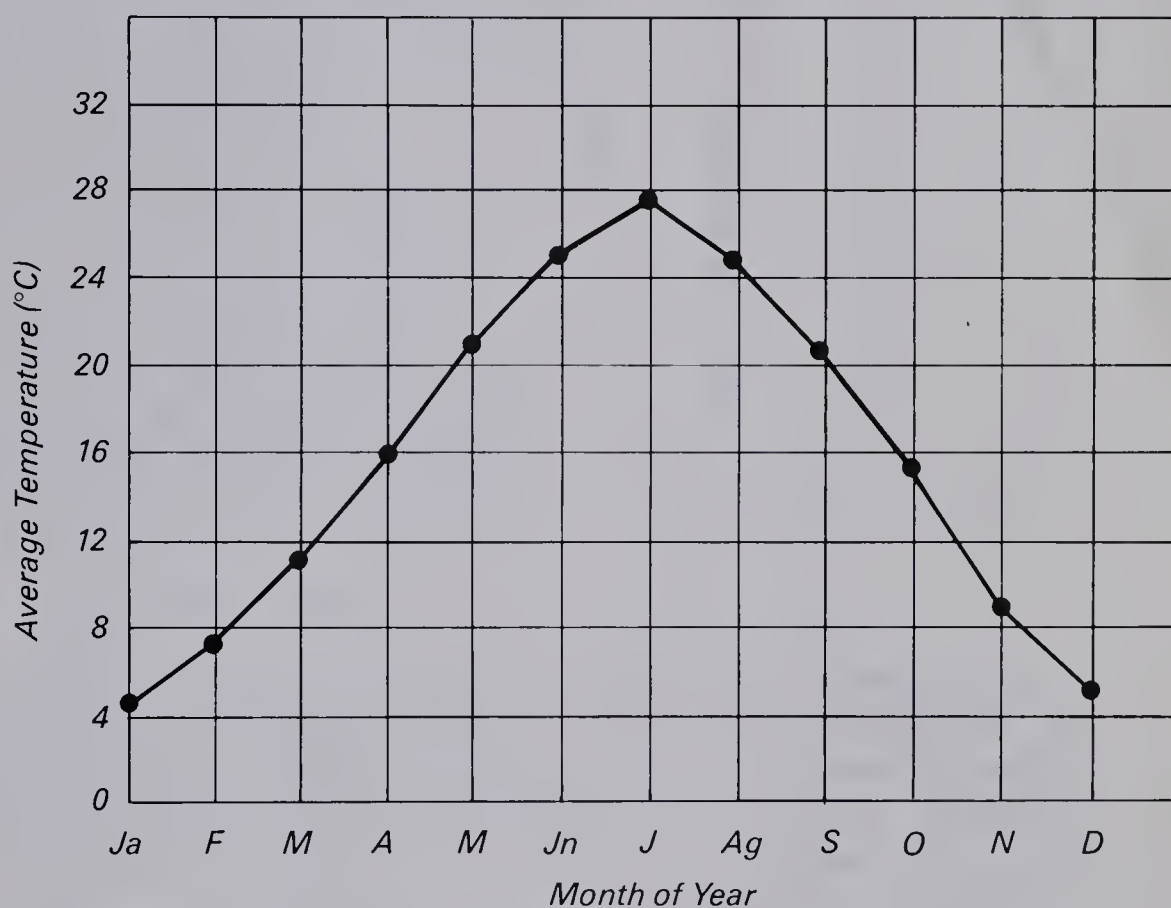
Data From Observations Made Over Time This kind of data also is often displayed on a line graph. A line graph would be used to show the annual temperature change in the Tennessee forest.

In graphs showing observations over time, time is marked along the X-axis. The thing being observed—the variable—is always marked along the Y-axis. Graph 2 of the data from Table 2 shows months on the X-axis, and the average monthly temperature on the Y-axis.

Table 2. Annual Temperature Changes in a Tennessee Oak-Hickory Forest

<i>Month of Year</i>	<i>Average Temperature (°C)</i>
January	5
February	7
March	11
April	16
May	21
June	25
July	27
August	25
September	21
October	15
November	9
December	6

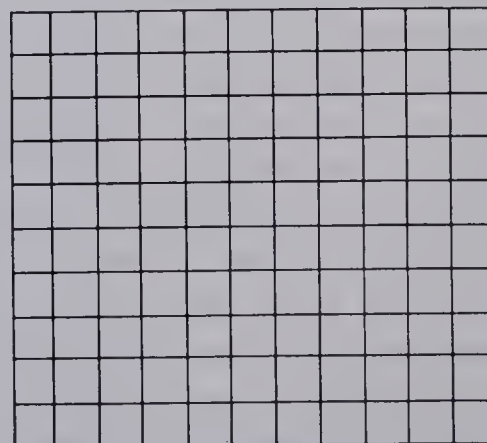
Graph 2. Annual Temperature Changes in a Tennessee Oak-Hickory Forest



2. Examine the data on the growth of bacteria in the following table. Prepare a line graph in the graphing space provided. Label the X and Y axes and mark the scales. Be sure to title the graph.

Growth of Bacteria

<i>Time (in minutes)</i>	<i>Number of Bacteria</i>
0	1
20	2
40	4
60	8
80	16

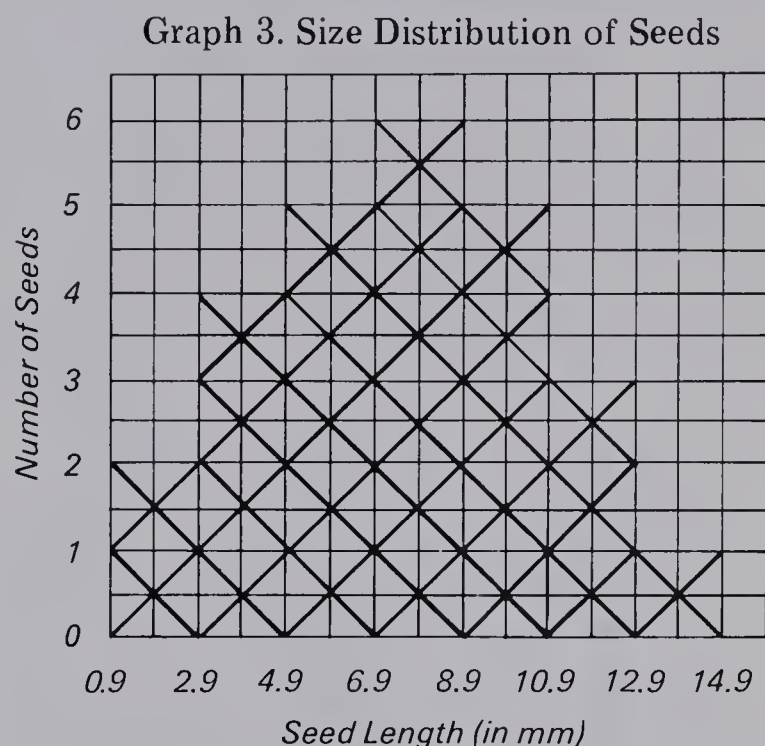


B. Making a Histogram

A histogram shows the frequency distribution of data, that is, how often each piece of data occurs. It is useful for graphing pieces of data with a wide range of values. For example, notice in Table 3 how much the lengths of seeds vary. The data on seed lengths can be displayed vividly in a histogram.

Table 3. Length of Seeds
(in mm)

2	6	10
1	5	9
3	7	10
4	7	10
4	8	11
3	7	12
5	7	11
5	8	14
6	9	



A histogram displays pieces of data grouped together in bars. Each bar contains at least one piece of data—usually it contains several pieces. The width of the bar represents the range of data in that group. The length of the bar shows the frequency, or how many pieces of data are in that group. For example, if a bar contains four pieces of data, it will be four units in length.

The scales showing the range of data in a group are marked along the X-axis. Each range has limits, from the lowest value of the data in the group to the highest value. For example, the range of seed lengths in the first bar of Graph 3 is from 0.9 to 2.9 mm. All seeds that are from 0.9 to 2.9 mm long belong in this group.

The scale of the X-axis should be uniform, just as in a line graph. You must design the scale so that each piece of data will fall *between* two marked lines—the limits of the range—and not on the lines. If you have data measured to the nearest tenth of a millimetre (0.1), mark the scale to the nearest hundredth (0.01). In Graph 3, the seeds were measured to the nearest millimetre. Consequently, the scales are marked to the nearest tenth; notice that each ends in 0.9.

The Y-axis of a histogram represents the number of data in each group. In the first bar of Graph 3, there are two pieces of data (one seed measuring 1 mm and one measuring 2 mm), so the bar is two units high. Mark an X on the graph each time a piece of data is added to a bar. As you assign a piece of data to a group, the bar grows one unit in length. Before beginning the graph, you might find it useful to make a brief table showing how many pieces of data fall into each group.

The most difficult part of making a histogram is determining the range for the X-axis. Here are a few guidelines. If you have many groups with only one or two pieces of data in each—or none at all—then your range is too small. If you have only a few groups with many pieces of data in each, then your range is too large. Try to set the range so that there will be either five or seven bars.

In Graph 3 we used seven bars. This is only one of the possible histograms that could be made from this data. The best way to understand the process is to make a histogram yourself.

3. Examine the data on femur lengths in grasshoppers in the following table. Make a histogram from this data. Label the X and Y axes, mark the scales, and title the histogram.

Femur Lengths
in Grasshoppers (in cm)

1.1	2.9
1.6	2.7
1.7	3.2
1.9	3.3
2.4	3.3
2.3	3.4
2.5	3.7
2.7	3.8
2.6	4.4
2.8	

ANALYSIS

4. You are doing an experiment involving the gain in mass of rats given different amounts of vitamins. When you make the graph of the data, you will place the independent variable on the X-axis. What is the independent variable in your experiment? Explain.

5. Two spaces on the X-axis of a line graph represent one unit of measurement. Must two spaces on the Y-axis also represent one unit?

6. What does the length, or height, of a bar on a histogram represent?

7. Explain how to set up the X-axis of a histogram.

8. You are observing a meadow over a period of time to find out if its population of bluejays is growing or decreasing. What kind of graph is best for displaying this data?

3 The Compound Microscope

PURPOSE

To learn the structure and basic use of the compound microscope.

MATERIALS

compound microscope

newsprint

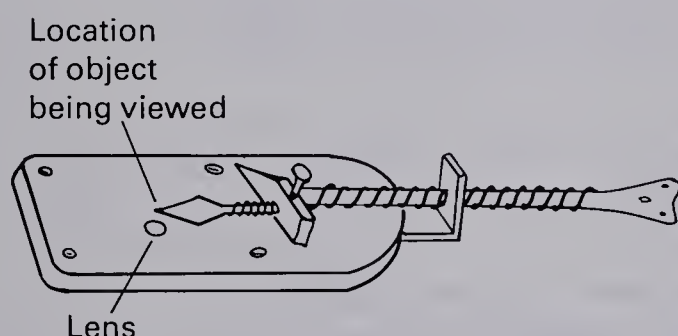
slides and coverslips

scissors

INTRODUCTION

The microscope is the biologist's basic tool. It has been developed to help explore the world of living things too small to be seen with the naked eye.

Early microscopes, like the one Antony van Leeuwenhoek made, had only one lens and were difficult to use. The biggest problem was magnification. The more powerful the lens—for greater magnification—the closer the viewer's eye had to be to the lens. At very high magnification, the lens almost touched the eye. The early microscope user had to be very steady indeed!



A major advance in microscopes came with the invention of the compound microscope. It has two sets of lenses, which magnify objects much more than a single lens.

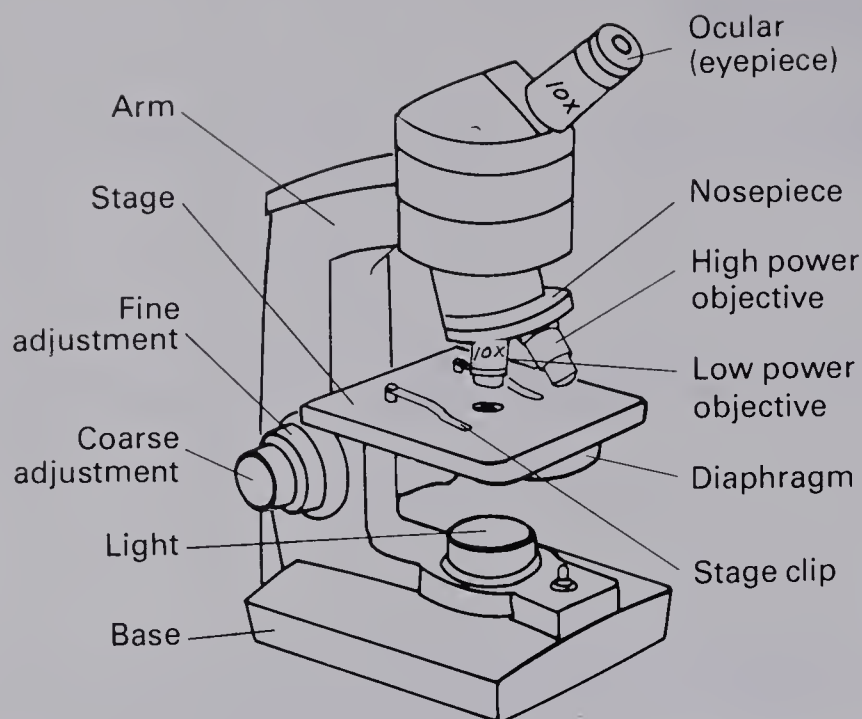
PROCEDURE

A. Structure of the Microscope

The compound microscope has four basic parts: the lens system, the focusing system, the stage, and the lighting system.

The Lens System One of the two sets of lenses are the objective lenses. They work much as did the lens of the early, simple microscope. The objective lenses make the initial or primary magnification. They are located in the nosepiece of the microscope.

Take Care: The compound microscope is a delicate instrument and can easily be damaged. Always carry it carefully with both hands, one under the base and the other holding the arm.



Compound Microscope

Inscribed on each objective is the magnification or power of that lens. This tells the number of times the lens magnifies the image. For example, if you are looking at a strand of hair with a 4X (four-power) lens, the hair will appear four times its actual size.

Your microscope probably has at least two objective lenses. Some microscopes have as many as four objectives. Rotate the lenses in the nosepiece until they click into position. The objective lens in use is always the one directly under the body tube. Usual powers for objective lenses are:

4X	This is the scanning lens.
10X	This is the low power lens.
40X	This is the high power lens.
100X	This is the oil immersion lens. This lens should not be used without special instructions from your teacher.

1. What is the power of each objective lens of your microscope?

-
2. Note the length of each lens. Is the higher power lens longer or shorter than the lower power lens?
-

The second kind of lens in the microscope is the ocular, sometimes called the eyepiece. This lens is located at the top of the body tube. The ocular serves as a small telescope, magnifying the image made by the objective lens. This enlargement is called the secondary magnifica-

tion. The magnification of the ocular may be 5X, 10X, 15X, or 20X. The most common power used in microscopes is the 10X ocular. Examine the ocular of your microscope. Do not remove it from the body tube. If the power is not stamped on the top portion of the ocular, you may assume that it is 10X.

The total magnification of the microscope is determined by multiplying the primary magnification (from the objective) by the secondary magnification (from the ocular). For example, if the objective lens is 10X and the ocular is 5X, the total magnification is: $10X \times 5X = 50X$.

3. Calculate the total magnification for each lens combination on your microscope. Show your calculation in the same form as in the example above.

-
4. Calculate the total magnification for the lens system in use on the microscope illustrated on page 12.
-

The Stage A specimen to be viewed through the microscope is mounted on a glass slide and covered with a coverslip. The slide rests on the stage, the flat surface beneath the body tube. Stage clips hold the slide in place. Also, they help in making slight adjustments in the slide's position by holding the slide steady.

The stage should always be kept in a horizontal position. If you tilt the stage, the specimen will slip to the bottom edge of the slide. Even with commercially prepared slides, the stage should be kept horizontal. A commercial slide can be ruined as the coverslip slowly slips downward on a tilted stage.

Both the slide and the stage are extremely smooth. Water between them acts like glue and causes the slide to stick to the stage. If water gets on the stage—STOP—and dry both the stage and the bottom of the slide with a paper towel before proceeding.

The Lighting System For you to see the specimen, light must pass through it and the lenses to your eye. The lighting system is located under the stage of the microscope. There are three different types of lighting systems.

The simplest system uses a concave mirror to focus a beam of light on the slide. Tilt the curved surface of the mirror to face a light source: room lights, windows, a desk lamp.

Another lighting system uses a lens under the stage to focus the light. If there is a substage lens and a mirror on your microscope, use the flat side of the mirror to reflect light through this lens.

A third lighting system uses a substage light instead of a mirror. If your microscope has a light, turn it on only when you are actually looking at the specimen. The light gets hot and can easily destroy your specimen.

Under the stage you will also find the diaphragm. It is used to

Caution: Do not use direct sunlight as a light source. Sunlight can harm your eyes.

adjust the amount of light that passes through the specimen. The diaphragm works like the aperture on a camera. Practice opening and closing the diaphragm while looking through the eyepiece. Notice how the amount of light increases and decreases.

The Focusing System In order to bring the image of the specimen into proper focus, it is necessary to change the distance between the slide and the objective lens. This can be done in one of two ways, depending upon the microscope you are using. Either the lenses can be moved or the stage upon which the slide rests can be moved.

Focus is controlled by two knobs. The coarse adjustment knob is for coarse focusing, and the fine adjustment knob is for fine focusing. Locate these on your microscope. Turn the coarse adjustment knob.

5. Do the lenses move up and down or does the stage move up and down?

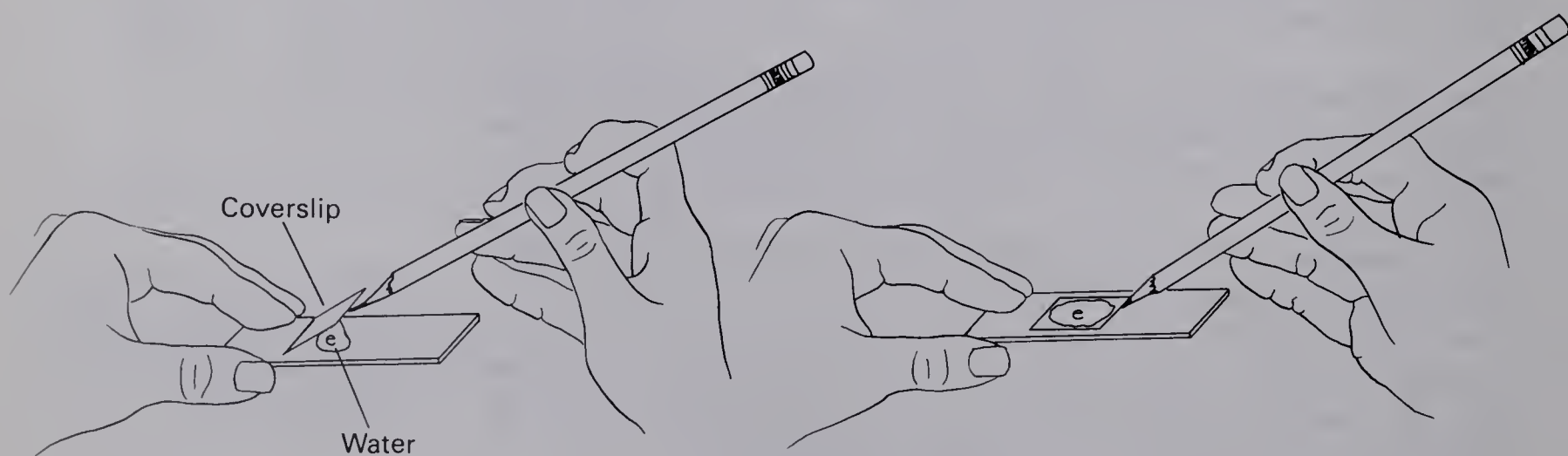
B. Using the Microscope

Put the low power objective in place. Look through the ocular and adjust the light so that you see a uniformly bright field of view. The field of view, also called the field, is that area you can see through the lens. If you see specks of dirt in the field, clean your lenses with lens tissue.

Now prepare a slide to view under the microscope. Cut a lowercase "e" from a newspaper and place it in the center of a clean slide. Put a drop of water on top of the letter. Next, place the edge of a coverslip against the water, and with a pencil gently lower the coverslip over the "e." Placing the coverslip in this manner prevents bubbles from forming. Be sure that the bottom of the slide is dry. This type of slide is called a wet mount.

Place the slide under the stage clips, so that the "e" is right side up. You are now ready to focus on the "e."

Take Care: If the lens is dirty or you get water on it, gently wipe it with lens tissue. Never use facial tissues. Lenses are made of soft glass and scratch easily.



Focusing always begins with the lower power (10X) objective. First, click the low power objective into position in the nosepiece. Then, looking at the side of the microscope, turn the coarse adjustment knob until the objective is as close as possible to the slide without touching it. Now look through the ocular and turn the coarse adjustment knob in the direction that will move the objective away from the stage. The "e" will come into approximate focus. To sharpen the focus, turn the fine adjustment knob back and forth.

Are you surprised that the borders of the letter "e" are far from perfect? Note the position of the letter through the microscope. The letter on the slide is right side up.

6. Is the position of the letter viewed through the microscope the same as it is on the stage?

Take Care: Be sure that the objective does not touch the slide—both the lens and slide can be damaged. Do not look through the eyepiece while *lowering* the objective toward the stage. It is difficult to judge through the eyepiece how far the objective is moving.

7. Draw the letter "e" as it appears through the microscope.

While looking through the microscope, move the slide to the right.

8. Which way does the letter appear to move when viewed through the microscope?

9. Push the slide away from you on the stage. Which direction does the letter appear to move when viewed through the microscope?

10. Summarize what you have just learned about apparent movement under the microscope.

Now, look at the "e" under high power. First, under low power, center the "e" in the field of view. Switch to high power by turning the nosepiece until the high power objective clicks into place. Sharpen the focus by turning the fine adjustment knob.

If you cannot find the "e" under high power, try this. Look through the ocular and move the slide slightly. If this does not bring the "e" into view, move the slide in other directions.

11. Draw the part of the "e" that you see under high power.

Take Care: Never use the coarse adjustment knob in high power. The objective is very close to the slide in high power, and coarse adjustment could cause the objective to hit the slide.

When you are finished using the microscope, remove the slide from the stage. Rinse the slide and coverslip with water. Dry the slide with a

paper towel—not lens tissue. Glass coverslips should be air dried to prevent breakage. Return both to their proper places. Finally, be sure the microscope is on low power and put it away. Remember to carry the microscope carefully, with one hand under the base and the other holding the arm.

ANALYSIS

12. What are the two kinds of lenses on a compound microscope? What does each do?

13. How do you increase the amount of light that passes through the specimen? How do you decrease the amount of light?

14. How do you determine the magnification of a microscope?

15. What is the relationship between movement on the stage and movement seen through the lenses?

16. Describe the procedure for focusing a microscope using coarse and fine adjustments.

FOLLOW-UP

- Observe an insect leg under low and high power. Can you see anything on the leg under high power that you did not see under low power? What?
- Observe several fabrics—wool, polyester, cotton, corduroy, etc.—under the microscope. What similarities and differences do you see?

4 Better Use of the Compound Microscope

PURPOSE

To improve microscope technique and learn how to measure objects with the compound microscope.

MATERIALS

compound microscope paper

slides and coverslips ruler

hair

INTRODUCTION

Learning to use the microscope is somewhat like learning to play a musical instrument. To play the guitar, you first learn how to position your fingers and produce single notes. Then you learn to put the notes together into chords to make music.

In the previous lab, you learned the basics of microscope use. In this lab you will learn the finer points, and how to measure objects with the compound microscope.

PROCEDURE

A. Adjustments With the Compound Microscope

Make a wet mount of two hairs crossed in an X position. Locate the X under low power. Position the slide so that the X is exactly in the center of the field and bring it into sharp focus. Switch to high power. If the hairs are in focus in the center of the field, the microscope is said to be parfocal. Parfocal means that only slight focusing changes are needed when objective lenses are rotated. The hairs can be brought into sharp focus with the fine adjustment knob.

1. Is the microscope you are using parfocal? Note any adjustments you must make to bring the hairs into proper focus under high power.

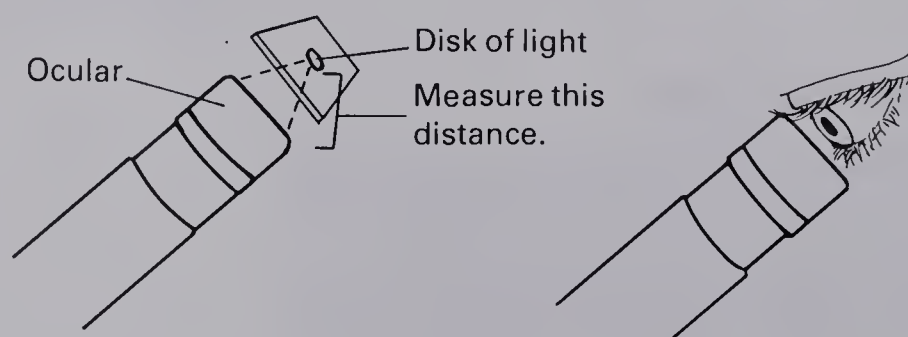
Next, adjust the light using the diaphragm under the stage. Close the diaphragm until you can clearly see the ridges or markings on the hair. This capability of the lens system to show fine detail is called resolution. At high resolution, the parts of the object are clear and distinguishable.

2. Draw a small portion of one of the hairs.

3. Focus the fine adjustment knob back and forth. Can you tell which hair is on top? How can you tell?

Put the lower power objective into position. Use the fine adjustment knob to bring the hairs back into sharp focus.

Rest a small piece of notebook paper flat against the ocular. Raise the paper slowly. Notice that light comes through the microscope onto the paper. Move the paper up and down until you see a small, sharply defined disk of light. With a ruler, measure the distance between the top surface of the ocular lens and the paper. This distance is known as the eye relief of the ocular.



4. What is the eye relief of your microscope? Make this measurement in millimetres.

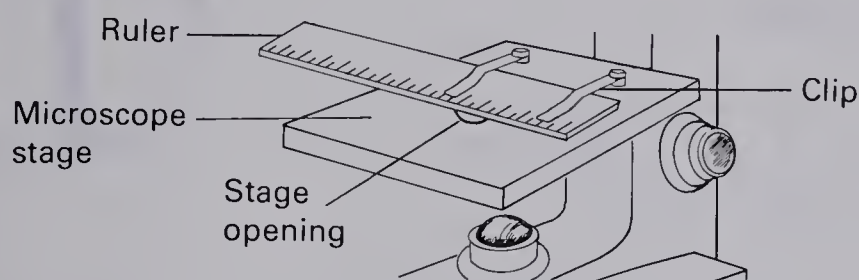
To see the full field, position your head so that the inside of your eye is the same distance from the ocular as the eye relief. If you wear eyeglasses, you will probably have to remove them in order to get your eye close enough to the ocular. You do not have to remove contact lenses. When looking through the ocular, keep both eyes open to avoid eyestrain.

B. Measurement With the Compound Microscope

The compound microscope is a versatile instrument. Not only is it used to magnify objects, but it can also be used to measure objects. The microscope is an especially valuable tool when you wish to determine the size of tiny specimens that are invisible to the naked eye.

With the 10X objective in place, put your ruler across the middle of the stage. Focus on the millimetre markings.

The unit usually used in microscopic measurement under the compound microscope is the micrometre (μm). The micrometre is one-thousandth of a millimetre.

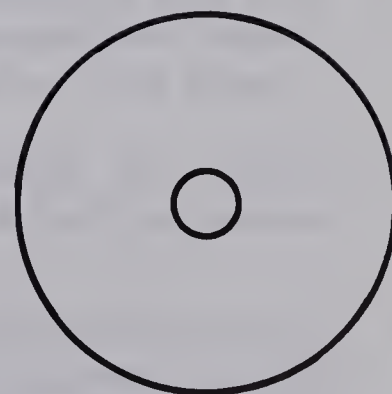


5. What is the diameter of the field of your compound microscope (in μm) with the 10X objective in place?

Rotate the nosepiece to bring the high power objective into position. Notice that the field of view is too small to be measured with your ruler. You can calculate the diameter of the field under high power in the following way. Divide the value of the high power objective by the value of the low power objective. For example, $40\text{X} \div 10\text{X} = 4$. Then divide the diameter of the low power field by this number. The resulting number is the approximate diameter of the high power field.

6. Calculate the diameter of the high power field of view on your microscope.

7. Examine the illustration. Assume the field diameter is $1000 \mu\text{m}$. Estimate the diameter of the small circle in the field.



ANALYSIS

8. Why should you know the eye relief of your microscope?

9. How can you adjust the resolution of your microscope?

10. When measuring with the microscope, why is it important to know the diameter of the field?

The following diagram shows the field of view under a compound microscope with a millimetre ruler across the stage. The ocular is 10X and the objective is 10X (low power).



11. What is the magnification at low power?

12. What is the diameter of the field of view (in μm)?

13. What is the magnification of this microscope using the 40X (high power) objective?

14. What is the diameter of the field of view under high power?

FOLLOW-UP

- If a stage micrometer is available, measure the diameter of the field of your compound microscope. How does your measurement compare with your answer to question 5?
- Measure the thickness of a human hair using the compound microscope. Find the approximate size of table salt crystals.
- List the eye relief distances of the oculars on all the microscopes in your classroom. Group them by similar kinds of microscopes. Find the mathematical average of these distances for each kind of microscope. Are some of the measurements quite different than others? Why might this be the case?

5 The Dissecting Microscope

PURPOSE

To learn how to use the dissecting microscope, and to learn how to measure objects with the dissecting microscope.

MATERIALS

table salt	leaves
dissecting microscope	newsprint
slide	ruler
2 dissecting needles	scissors

INTRODUCTION

Have you ever had trouble removing a sliver from your finger? A dissecting microscope would have made the job easier. It is a very useful tool when you are manipulating and measuring small objects.

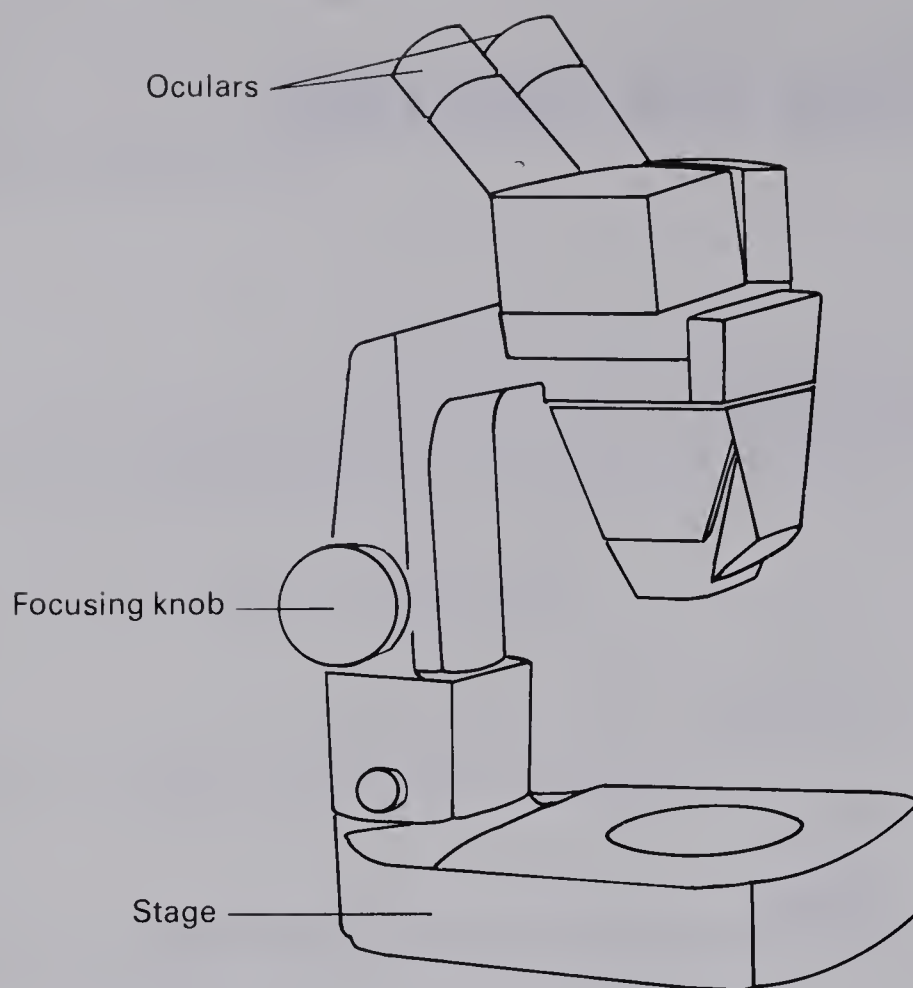
PROCEDURE

A. The Dissecting Microscope

The dissecting microscope has objective and ocular lenses as does the compound microscope. The basic difference between the two kinds of microscopes is that the lenses of the dissecting microscope are designed to be used at a much greater distance from the specimen, and they have much lower magnification. Consequently, the dissecting microscope has a field of view several times larger than that of the compound microscope. For example, with a compound microscope you can just about fit the eye of a mosquito into the field of view at low power. With a dissecting microscope you can fit several mosquitoes into the field.

Many dissecting microscopes are stereoscopic, which means that they have two complete sets of lenses. Each set of lenses produces a separate image. Because you look through the dissecting microscope with both eyes, you get a three-dimensional view of the object on the stage.

Examine the oculars of your stereoscopic dissecting microscope. One of the oculars (usually the left one) is adjustable. You should focus your microscope with the knob for the ocular that is *not* adjustable. Note that the focusing knob does not have a fine adjustment control. The focusing adjustments are not as critical as on the compound microscope.



Dissecting Microscope

Next, fine tune the adjustable ocular to suit your vision—it compensates for differences in your two eyes. The oculars also move sideways, allowing you to adjust them for comfortable viewing with both eyes.

Most objects you will view with the dissecting microscope are opaque. Light does not pass through an opaque object. To be visible, it must be illuminated from above. Light then reflects from the object back into the lenses. Some dissecting microscopes also have a light source underneath the stage. This is used when the specimen is transparent.

Cut a lowercase “e” from a newspaper. Place it on a slide without water or a coverslip. Center the slide on the microscope stage. Bring the “e” into focus, making the two necessary adjustments of the oculars.

1. What are the two adjustments that you made with the oculars?

2. How does the position of the “e” viewed through the microscope compare with the position of the “e” on the slide?

3. Move the slide on the stage while looking through the microscope. How does the direction of the actual movement compare with the direction of movement seen through the microscope?

Name _____ Date _____

4. Put a few table salt crystals in a dish and view them under the microscope. What geometric shape best describes the salt crystals?

Next, examine the three leaves you brought to class. Note the structures of each leaf.

5. Draw the three leaves, including all the details you can see.

Now examine each leaf under the dissecting microscope. You will see the structure in finer detail.

6. Draw the three leaves as they appear under the dissecting microscope.

To get the feel of working under the dissecting microscope, choose the leaf with the largest veins to dissect. Using two dissecting needles, dissect a vein out of the leaf. Move the vein around with the needles to get a more complete view of its structure.

B. Microscopic Measurement

Place a ruler across the middle of your dissecting microscope. Bring the ruler markings into focus.

7. What is the diameter of the field (in mm)?

If your microscope has more than one power, change the power and measure the field again.

8. What is the diameter of the field under the second power (in mm)?

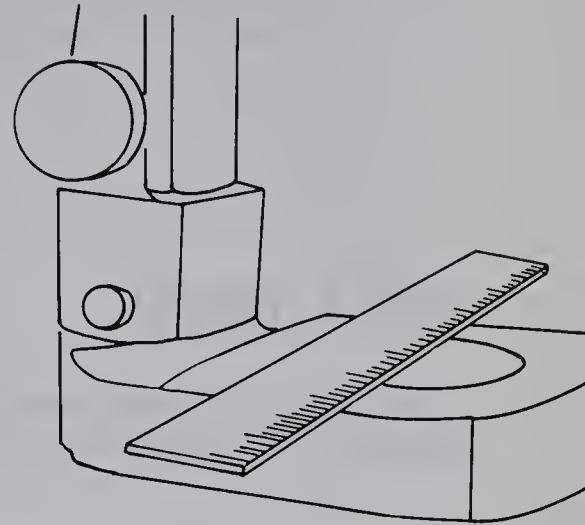
9. As the magnification of the microscope increases, what happens to the diameter of the field of view? Does the diameter remain the same, increase, or decrease? Try to answer this question even if your microscope has only one power.

The information about the diameter of the field can be used to make approximate measurements of objects viewed under the microscope. For example, if the diameter of the field is 4 mm and the object goes halfway across the field, the object is 2 mm in size.

ANALYSIS

10. Why is it desirable for a dissecting microscope to have lower magnification than a compound microscope has?

11. How does movement of specimens under the dissecting microscope compare with movement under the compound microscope?



12. What must be the position of a light source to illuminate an opaque object? a transparent object?

13. When measuring with the microscope, why is it important to know the diameter of the field?

FOLLOW-UP

Line up salt crystals under the dissecting microscope with a dissecting needle or probe to form the capital letter "E."

6 The Dichotomous Key

PURPOSE

To learn how a dichotomous key is constructed and to become proficient in using a key.

MATERIALS

paper

pencil

INTRODUCTION

Classification of organisms helps biologists identify and understand the co-inhabitants of the earth. However, the information must be organized for easy use if it is to be of any value. Can you imagine trying to use a dictionary in which the words are not listed in alphabetical order?

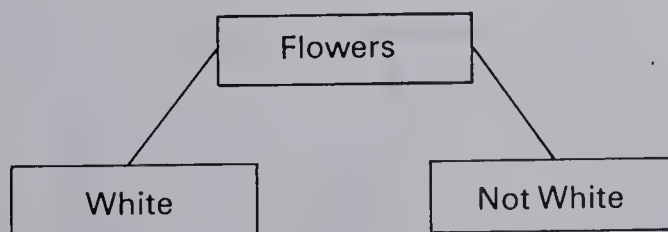
In biology we frequently want to know what kind of organism we have found. Among the best classification aids are dichotomous keys. Perhaps you have used a dichotomous key to help identify birds or flowers.

A beginner may just flip through the key, randomly looking at the pictures. Many birds and flowers have been successfully identified in this manner. However, there is a high risk of error. You certainly would not want to use the random hit-or-miss method to identify edible mushrooms. Such a method is not accurate enough to detect certain details, which may be important in distinguishing between similar species.

PROCEDURE

A. Using a Dichotomous Key

Using a dichotomous key is easy if you understand how the key is constructed. Dichotomous means “divided into two parts.” The key is designed to divide a group of organisms into two smaller groups. Example:



Each group is subdivided into two more groups, and so on until each individual species stands alone.

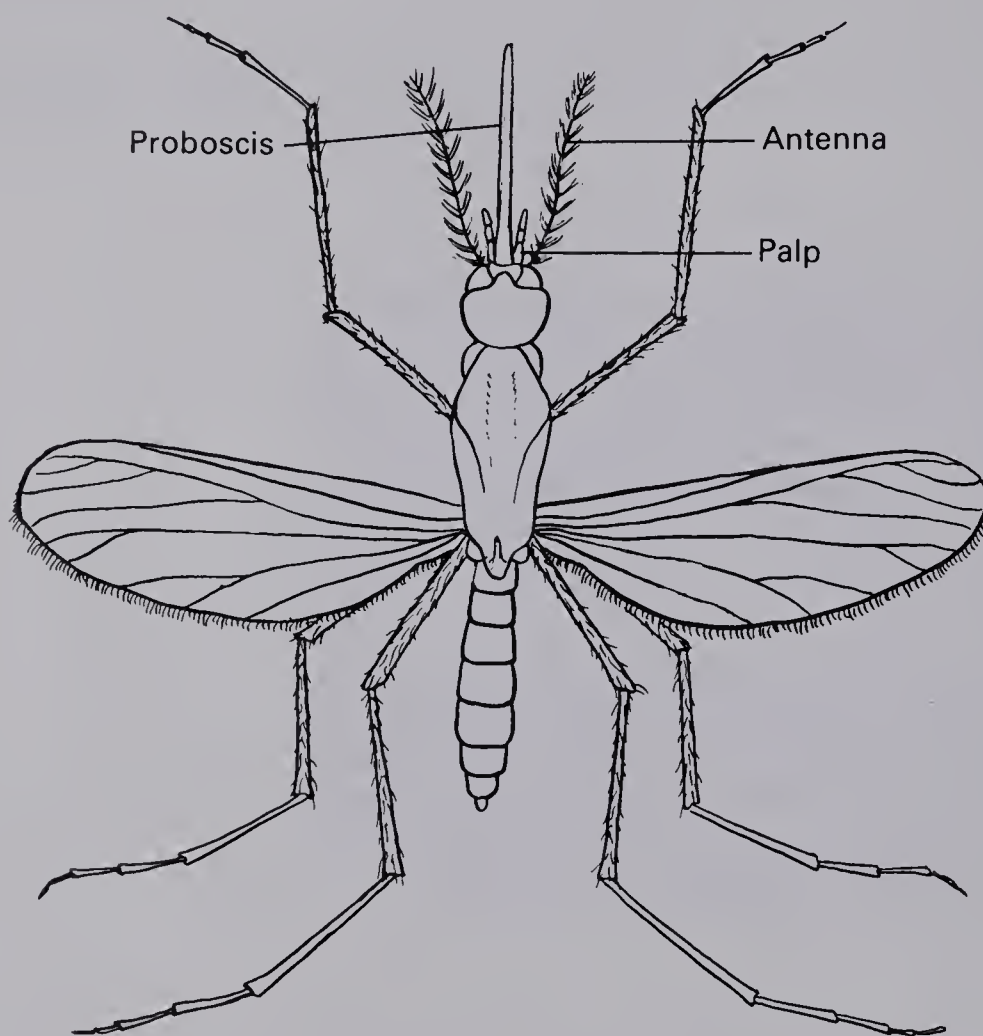
At each step in the key you are faced with two alternatives. The choice you make eliminates some members of the group. Finally, when

you have eliminated all the other members in the group, you have isolated one organism. This should be the organism you are looking for.

Below is an example of a dichotomous key. This key is for five genera of female mosquitoes. Since only female mosquitoes bite, they are usually of greater interest than males.

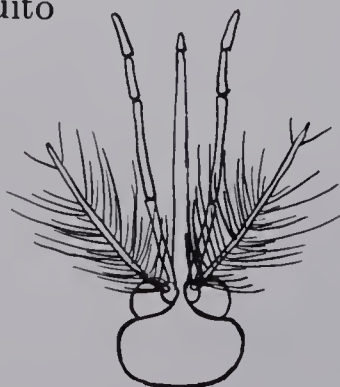
Read through the key. Notice how the numbers are arranged: there are always two alternatives. Each time you make a choice, you are directed to the next step. The groups are arranged so that the user of the key will have little difficulty in making the proper choices.

After you have studied the key to see how it is constructed, use it to identify the mosquito in the diagram. In the space provided, write the number of the choice you took at each step, and then give your identification of the mosquito.

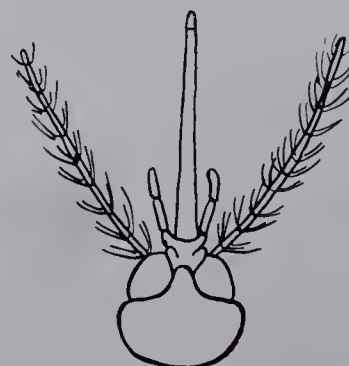


Dichotomous Key of Female Mosquitoes

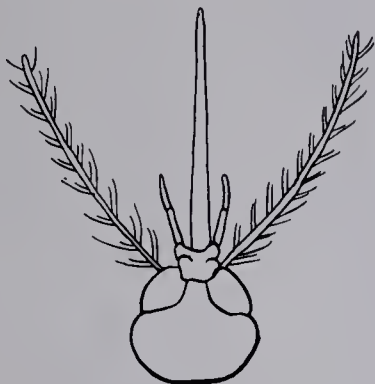
- 1a. Antennae very bushy—
Male mosquito



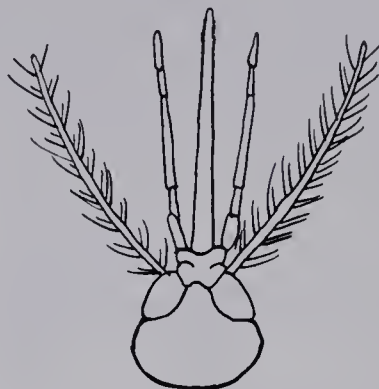
- 1b. Antennae not bushy—
Go to 2



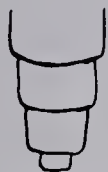
2a. Palps much shorter than proboscis—Go to 2



2b. Palps as long as proboscis—
Female *Anopheles*



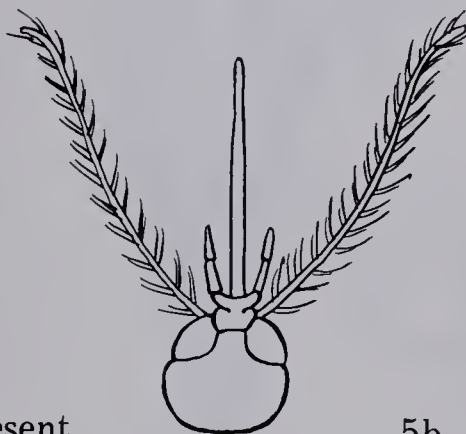
3a. Tip of abdomen blunt, without points—Go to 4



3b. Tip of abdomen with points—Go to 5



4a. Antennae much longer than proboscis—
Female *Deinocerites*



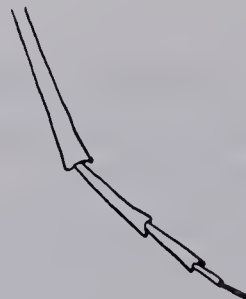
4b. Antennae shorter than proboscis—Female *Culex*



5a. Many long scales present on hind legs—
Female *Psorophora*



5b. Hind legs without long scales—Female *Aedes*



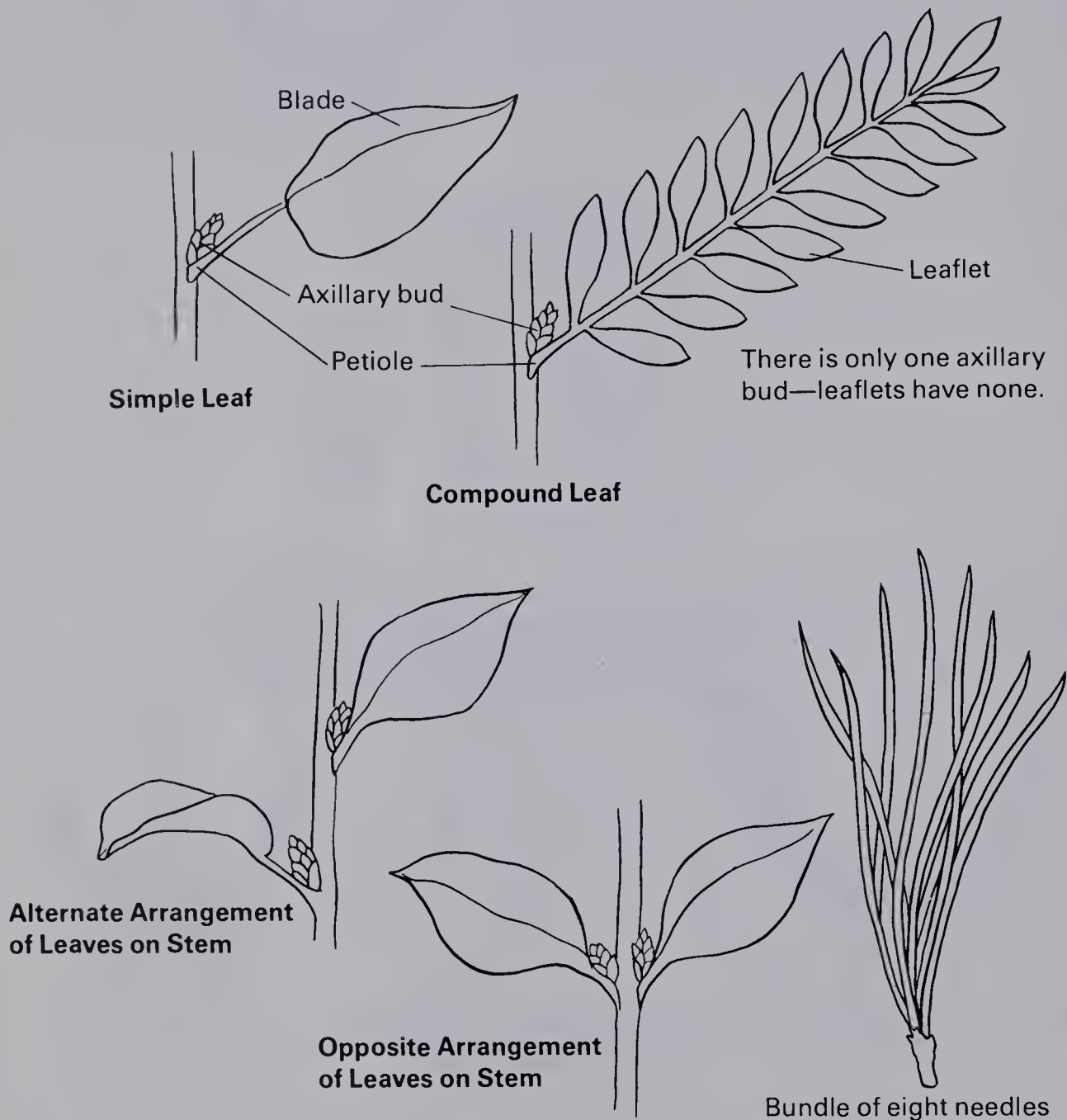
1. _____
Numbers of choices

2. _____
Genus of mosquito in diagram

B. Constructing a Dichotomous Key

A good way to learn how to use a dichotomous key is to make one. You will construct a dichotomous key of tree leaves.

The drawings contain information about the general structure of leaves and their arrangement on stems. First, study these drawings to become familiar with the general structure of leaves. Once you are familiar with the terminology, proceed to construct your dichotomous key.



Ten different kinds of leaves from various trees are shown on page 31. Examine the drawings to become familiar with the differences and similarities among the leaves.

Construct a dichotomous key using all ten leaves pictured. Begin making your key by finding characteristics of the leaves that appear in only two forms. For example, are the leaves opposite each other on the stem, or do they alternate? Set up your key like the one shown for the mosquito.

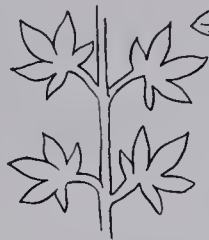
Remember—there is no *one* right key. Several different keys can be made with these leaves. The important thing is that your key works and is useful. When you have finished making your key, ask a friend to try to use it!



Jack Pine



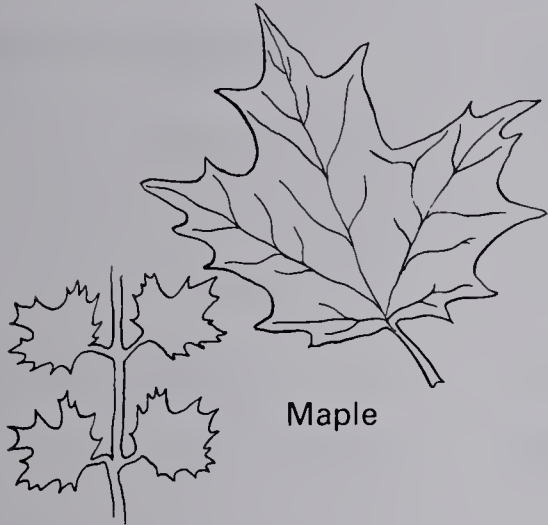
White Pine



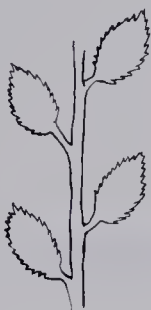
Buckeye



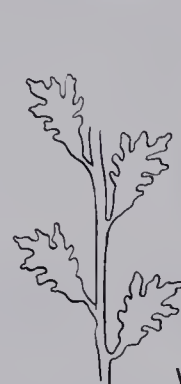
Horse Chestnut



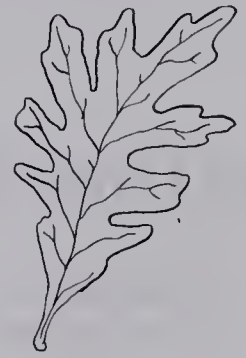
Maple



Elm



White Oak



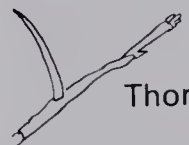
Pin Oak



Gingko



Hawthorn



Thorn

ANALYSIS

3. On the dichotomous key of female mosquitoes, what characteristics did you look at to identify the mosquito?

4. How many alternatives are there in each step of a dichotomous key?

5. Why is it important to look closely at the details of an organism when trying to identify it with a dichotomous key?

FOLLOW-UP

- Construct another dichotomous key of the leaves shown, different from the first one you made.
- What is the smallest number of steps needed in a key that will identify all ten types of leaves?

7 Protozoa

PURPOSE

To become familiar with some common protozoa.

MATERIALS

pure cultures of *Ameba*,
Paramecium, and *Chilomonas*

methyll cellulose solution

dropping pipette

compound microscope

slides and coverslips

INTRODUCTION

Protozoa can be found in almost any watery environment. They are one-celled organisms that belong to the protist kingdom.

Protozoa are consumer protists—they obtain food by eating other living things. Many protozoa move about a great deal. In fact, the way a protozoan moves is a key factor in its classification.

In this lab you will observe three common protozoa: *Ameba*, *Paramecium*, and *Chilomonas*.

PROCEDURE

A. Ameba

As an ameba moves, it constantly changes shape. This is because it moves by thrusting forward parts of its body. These footlike extensions are called **pseudopods**, which means “false feet.” The pseudopods surround food particles and bring them into the ameba, where they are digested.

Make a wet mount of the *Ameba* culture using a clean slide. The procedure for making a wet mount is in the Compound Microscope Lab, page 14.

Observe an ameba under low power with the compound microscope. Note its changing shape as it moves. As you watch the ameba, estimate its diameter in micrometres. Use the technique presented in the lab on microscopic measurement, page 19.

1. Draw an ameba. Label the pseudopods and give the size of the ameba in micrometres.

B. Paramecium

The oval-shaped paramecium is covered with small hairlike projections called cilia. By beating the cilia, the organism propels itself through the water. The paramecium, like other protozoa, normally travels in the direction of its anterior, or head, end.

The groove in the side of the paramecium is called the oral groove. Cilia push food to the end of the oral groove, where it is taken into the organism and digested.

Some paramecia have a star-shaped structure that seems to pulsate. This structure acts as a water pump, getting rid of excess water that enters the organism. The structure is called a contractile vacuole. Water is carried through channels (the points of the star) to the round contractile vacuole in the center of the star. The pumping action expels the water from the body.

On a clean slide, make a ring of methyl cellulose slightly smaller than the size of the coverslip. Use a dropping pipette to place a drop of *Paramecium* culture inside the ring. Wait one minute before placing the coverslip on the slide. This time allows the methyl cellulose and water to mix. The methyl cellulose will slow the paramecia, making them easier to observe.

Observe a paramecium under low power with the compound microscope. Note how it twists as it swims through the water.

2. Draw a paramecium and estimate its length in micrometres. Label the oral groove and the cilia. If you can find the contractile vacuole, sketch it onto your drawing and label it. Draw an arrow by the paramecium pointing toward its anterior end.

C. Chilomonas

The chilomonas is usually present in polluted water—it can be found in almost any pond where there is decomposing vegetation. This tiny organism often serves as food for amebas, paramecia, and other large protozoa.

The chilomonas is shaped like a long oval. It propels itself through the water with whiplike flagella, which are attached to its anterior end.

Make a wet mount of the *Chilomonas* culture, using methyl cellulose to slow the organism. Follow the procedure described in part B.

Observe a chilomonas under low power with the compound microscope. Watch the way that it uses its flagella for propulsion. Do the flagella work like an airplane propeller, pulling the chilomonas through the water? Or, do the flagella trail behind its body, pushing it ahead?

3. Draw a chilomonas and estimate its length in micrometres. Label the flagella. Draw an arrow by the chilomonas pointing toward its anterior end.

ANALYSIS

4. How does the shape of an ameba differ from the shape of a paramecium and a chilomonas?

5. Which is the slowest moving of the three protozoa you observed? Why might it be the slowest?

6. How does an ameba take in food? a paramecium?

7. In what way do many amebas and paramecia depend on chlo-
monas?

8 Algae

PURPOSE

To become familiar with four common algae.

MATERIALS

pure cultures of <i>Oscillatoria</i> , <i>Spirogyra</i> , <i>Euglena</i> , and diatoms	compound microscope
	slides and coverslips
methyl cellulose solution	dropping pipette

INTRODUCTION

Algae is the name given to a rather diverse group of organisms. Algae have several things in common. They contain chlorophyll and carry out photosynthesis. They lack vascular tissue. They do not have roots, stems, or leaves as do plants.

Many algae produce a colony of cells in the form of a strand called a filament. Each cell in the filament is capable of surviving on its own and forming a new filament.

PROCEDURE

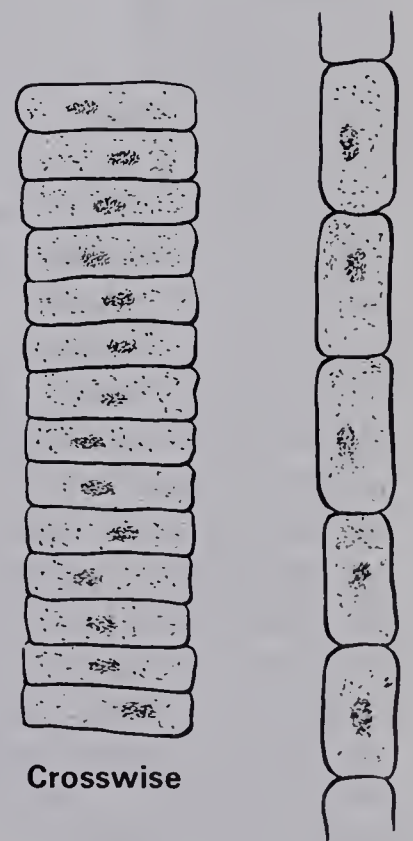
A. *Oscillatoria*

Oscillatoria is a member of the most primitive phylum of algae, known as blue-green algae. These algae have no nucleus; instead, the chromosome material is found throughout the cytoplasm. Because of these features, blue-green algae are prokaryotes and are classified in the moneran kingdom. The cells of blue-green algae appear blue-green because they contain a blue pigment in addition to the green pigment chlorophyll.

Blue-green algae are often found in polluted water, giving the water a foul odor. The cells are coated with a jelly-like material, which gives the algae a slimy appearance.

Oscillatoria is the most common of the blue-green algae. The genus is so named because its members are capable of making a swaying, or oscillating, movement. In reproduction, portions of a filament break off and grow into new filaments.

Make a wet mount of the *Oscillatoria* culture. View it under high power with the compound microscope. Examine the filament.



Crosswise

Lengthwise

The cells of the filament can be oriented either crosswise or lengthwise, depending on the alga.

1. Are the cells of the filament oriented crosswise or lengthwise?
-

On one of the filaments, locate the end that has a rounded tip.

2. Draw the rounded end cell and two or three adjacent cells. Estimate the size of the cell. Label the cell wall and the cytoplasm.

B. Spirogyra

Spirogyra is a member of the phylum known as green algae. It is frequently found in scum on the surface of a pond. Green algae are eukaryotes, having a nucleus. They also have chloroplasts and other organelles in each of their cells. These algae have the same kind of chlorophyll found in all of the higher plants, which include mosses, ferns, trees, and flowers. For this reason they are thought to be closely related to the higher plants and are classified in the plant kingdom.

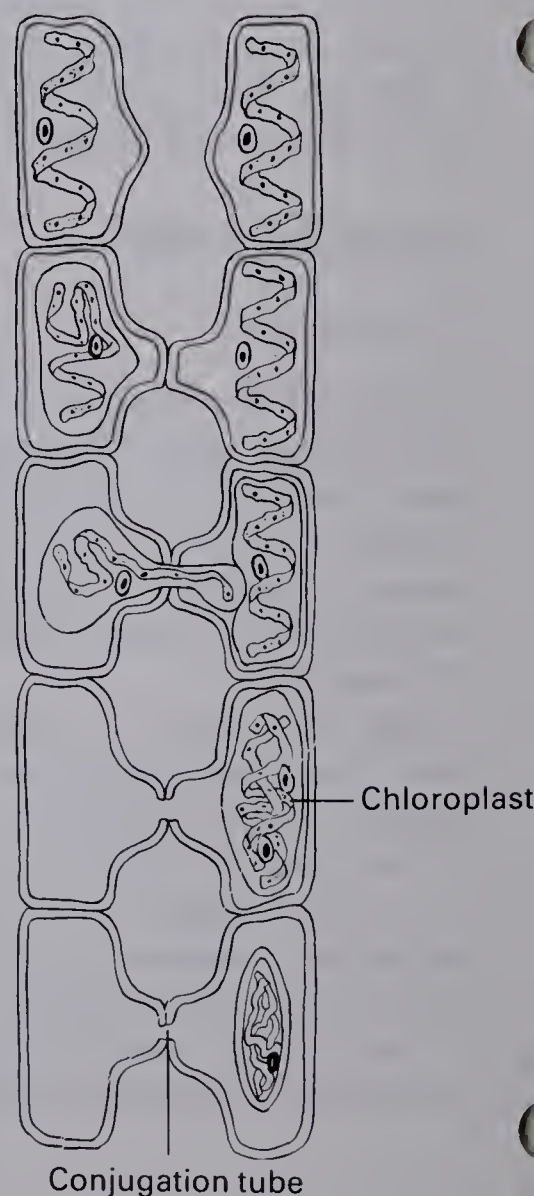
Spirogyra is named for the shape of its chloroplast. The chloroplast looks like a long ribbon that spirals around the cell from one end to the other. On the chloroplast are circular spots. These are starch storage areas called pyrenoids.

Spirogyra reproduces sexually by a process called conjugation. Tubelike extensions grow out of cells from two adjoining filaments. When the extensions touch, they fuse to form a tube connecting a cell in one filament with a cell in the adjacent filament. These tubes are called conjugation tubes. The cytoplasm and the nucleus from the cell in one filament migrate through the conjugation tube into the cell in the other filament. The union of the living material from these two cells is sexual reproduction.

This is a rather unusual form of sexual reproduction. What causes the tubes to form between only certain filaments? What determines which cell will migrate in which direction? These questions remain a biological mystery.

Make a wet mount of the *Spirogyra* culture and observe it under high power with a compound microscope.

3. Are the cells of the filament oriented crosswise or lengthwise?
-



4. Draw two cells of a filament and estimate their size. Label the cell wall, nucleus, chloroplast, and pyrenoids. If time permits, try to find some cells that are undergoing conjugation. Sketch the cells with their conjugation tubes.

C. Diatoms

Diatoms are probably the most abundant kind of algae. They are eukaryotes and belong to the phylum known as golden-brown algae, of the protist kingdom.

Most diatoms are single-celled organisms; however, some form filaments. They occur in a variety of shapes, such as boat-shaped, round, triangular, and rectangular. Some diatoms are multicellular, and take the form of filaments.

A diatom cell contains a cell wall, a nucleus, and several chloroplasts. The cell wall is unusual: it contains silica, the major component of glass. This makes the cell walls glasslike.

With the chlorophyll in the chloroplasts, diatoms produce food by photosynthesis. They convert the food to oil droplets, which they store in their cytoplasm.

Diatoms are so abundant in sea water that they provide much of the food eaten by aquatic animals. Because they are so numerous, they play an important role in replenishing the earth's oxygen supply.

When diatoms die, their glassy cell walls remain and accumulate on the bottom of the sea. These deposits may become hundreds of feet thick. Such deposits are called diatomaceous earth. This is mined and used commercially as abrasives, filtering agents, and insulation.

Make a wet mount of a culture of diatoms and examine it under high power with a compound microscope. The cell walls of most diatoms have distinctive markings. The quality of optical equipment is sometimes measured by how well it can resolve these markings.

5. Draw two diatoms and estimate their size. Show the markings on the cell wall. If you were able to distinguish the nucleus and the chloroplasts, draw and label them.

D. Euglena

Euglena are eukaryotes and belong to the phylum Euglenophyta of the protist kingdom. Euglena are unusual because they are like animals in some ways and like plants in other ways. The unique characteristics make euglena difficult to classify.

Sometimes euglena are classified as protozoa because of their animal-like characteristics. They lack a cell wall and can absorb nutrients from their surroundings. They move around a great deal, propelling themselves with whiplike flagella.

Sometimes euglena are classified as algae because of their plantlike characteristics. They have chloroplasts containing chlorophyll and so are able to perform photosynthesis. Here, since euglena produce their own food, they are considered producer protists and are grouped with the algae.

Make a wet mount of the *Euglena* culture. To slow the euglena, apply a ring of methyl cellulose slightly smaller than the coverslip. Then place a drop of the culture inside the ring and wait one minute before adding the coverslip.

Observe a euglena under the compound microscope. Note the small, green, diskshaped chloroplasts and the flagellum. You should be able to determine whether the flagellum pushes or pulls the euglena. In other words, does the flagellum pull from the anterior end like a propeller on an airplane, or does it push from the posterior end?

6. Draw a euglena and estimate its size in micrometres. Draw and label the flagellum, chloroplasts, nucleus, and cell membrane. Place an arrow by the euglena pointing toward its anterior end.

ANALYSIS

7. Where is chlorophyll found in *Oscillatoria*, *Spirogyra*, *Euglena*, and diatoms?

8. Why is *Spirogyra* classified in the plant kingdom?

9. Which alga is named for its form of movement? Which is named for the shape of its chloroplast?

10. How are diatoms useful to aquatic animals? to humans? to all life on earth?

11. What do euglena have in common with other algae?

12. The four algae included in this lab belong to four different phyla. Name the kingdom to which each alga belongs.

9 Microorganisms in Pond Water

PURPOSE

To observe microorganisms in pond water and be able to identify them.

MATERIALS

methyl cellulose solution

compound microscope

pond water

slides and coverslips

jar

dropping pipette

INTRODUCTION

A drop of pond water is teeming with life too small to be seen without a microscope. In this lab you will examine pond water to discover what types of organisms live there. Some organisms live near the surface of the water, others live near the bottom, and still others are distributed throughout the water.

While observing pond water you are likely to see protozoa, algae, and tiny invertebrates. Some of the most common inhabitants of pond water include *Ameba*, *Paramecium*, *Chilomonas*, *Oscillatoria*, *Spirogyra*, and *Euglena*.

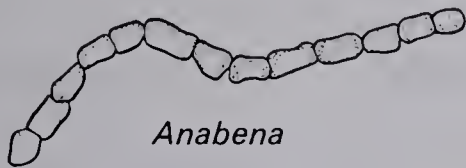
PROCEDURE

Prepare a wet mount of pond water and observe it under low power with the compound microscope. If some of the organisms are moving too fast for you to see clearly, prepare a new slide with methyl cellulose to slow them. Place a ring of methyl cellulose, slightly smaller than the coverslip, on the slide. Add a drop of pond water, and wait one minute before adding the coverslip.

Refer to the chart to help in identifying the organisms you observe. In your identification, look for such structures as nuclei, chloroplasts, cell walls, cell membranes, vacuoles, cilia, flagella, and pseudopods.

1. Sketch the most common organisms you find in the pond water. Include enough detail so that you will be able to recognize the same organisms the next time you see them. Include the name and estimate the size of each organism you sketch.

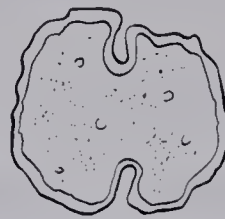
Algae



Anabena



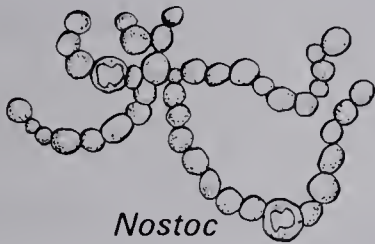
Chlamydomonas



Cosmarium



Euglena



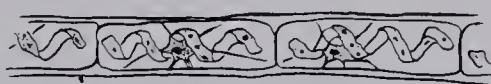
Nostoc



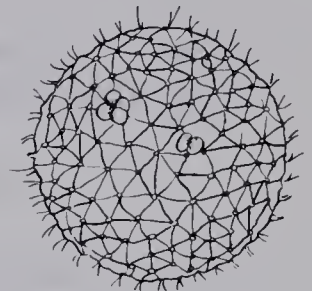
Oedogonium



Oscillatoria

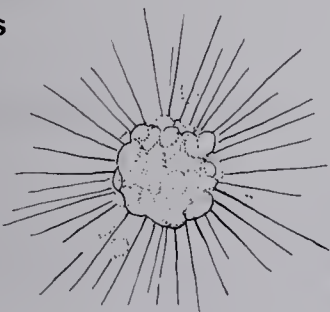


Spirogyra

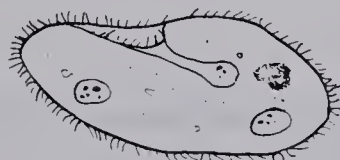


Volvox

Protozoans

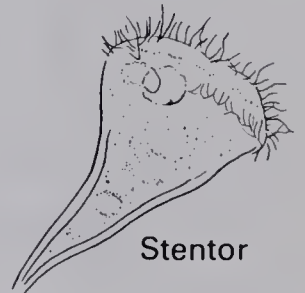
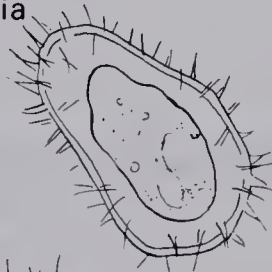


Actinosphaerium



Paramecium

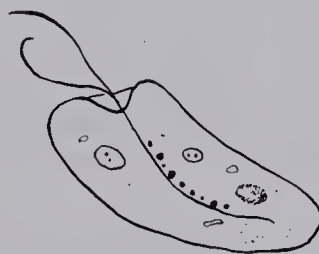
Stylonychia



Stentor



Colpidium



Chilomonas



Vorticella

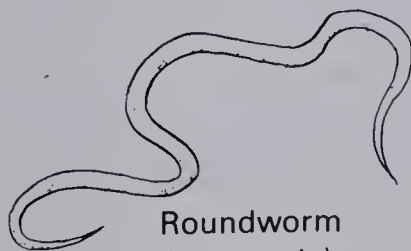


Ameba

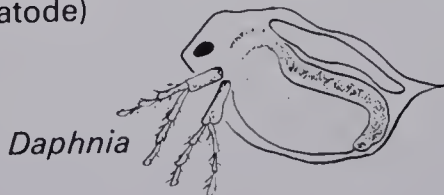
Invertebrate Animals



Rotifer



Roundworm (nematode)



Daphnia



Tubifex (annelid worm)



Cyclops

Common Pond Water Microorganisms

ANALYSIS

2. How many different types of organisms did you find in the pond water?

3. Did you find any organisms you have studied in previous labs? If so, which?

4. Which of the organisms you sketched move by means of cilia? of flagella? of pseudopods?

5. Which of the organisms you sketched are able to perform photosynthesis? How can you tell?

6. Which of the organisms you sketched are producers and which are consumers?

10 Fungi

PURPOSE

To become familiar with some common fungi.

MATERIALS

bread mold	compound microscope
mushroom fruiting body	dissecting microscope
yeast culture	slides and coverslips
wetting agent	forceps

INTRODUCTION

Did you know that if you have athlete's foot you are growing a fungus? The fungi include yeasts, mushrooms, and molds. Some fungi, such as certain wild mushrooms, contain substances that are poisonous. Other fungi add to the food supply. Cultivated mushrooms are considered an edible treat, cheese is produced by a fungus, and yeast is used in making bread and alcoholic products. Fungi are also important to the pharmaceutical industry in making antibiotics. Penicillin, for example, is produced by a blue-green mold.

Fungi have no chlorophyll and thus cannot make their own food. They grow on other organisms and consume organic material produced by these organisms. The fungi can be either parasites (such as athlete's foot), which grow on living organisms, or saprophytes, which grow on dead organisms.

The cell structure of fungi differs from that of plants and algae in several ways. Most importantly, the cell walls of fungi are made of chitin, the same material that forms the hard exoskeleton of insects. Also, the cells of many species of fungi have more than one nucleus.

Biologists who study fungi are called mycologists.

PROCEDURE

A. Bread Mold

A common type of mold is black bread mold. You may have seen it on bread, onions, or other foods.

The bread mold consists of a mass of white threads called a mycelium. An individual thread of the mycelium is called a **hypha**. Each hypha is an individual cell, and most hypha cells have more than one nucleus.



There are three kinds of hyphae in bread mold. Rhizoids are rootlike hyphae that grow beneath the surface of the bread. Sporangiphores are upright hyphae that grow above the bread's surface. They bear sporangia, which look like golf balls on tees. Sporangia produce tiny round spores, which are reproductive cells that can develop into new individual fungi. Like seeds, spores can withstand harsh conditions and begin growing when conditions are favorable. Stolons are horizontal hyphae that form connections between sporangiphores.

Put a small amount of bread mold on a slide and examine it under the dissecting microscope.

1. Draw the hyphae and the sporangia. Label the sporangia, sporangiphores, spores, stolons, and rhizoids.

Remove a few of the sporangia with a pair of forceps. Place the sporangia in a drop of wetting agent on a clean slide and cover with a coverslip.

Wetting agent is water with a drop of detergent. The spores are so light that they float on the surface of pure water and do not get wet. Wetting agent breaks the surface tension of the water, enabling the spores to sink beneath the surface.

Observe the spores under high power with the compound microscope.

2. Draw several spores. Estimate their size and color.

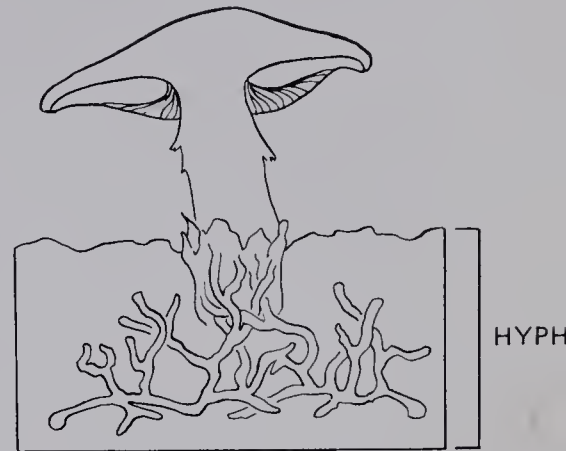
B. Mushrooms

The part of a mushroom that appears above the ground is only a small portion of the whole organism. An extensive underground network of hyphae forms before the familiar mushroom is produced.

The above-ground portion of the mushroom is known as the fruiting body. It consists of a round cap sitting on top of a stemlike stalk; both are made of many hyphae packed tightly together. On the underside of the cap are the gills. Gills are rows of hyphae that usually radiate out from the center of the cap like spokes of a wheel. The gills contain spore-producing structures called basidia.

Observe the fruiting body of a mushroom. Identify the stalk, the cap, and the gills.

3. Draw a mushroom fruiting body and label the three parts.



Remove the stalk from the cap. Place the cap, gills down, on a sheet of white paper. Cover the cap with a second piece of paper. Place the papers and the mushroom cap in a drawer overnight. Spores released from the basidia will fall onto the bottom paper, making a spore print.

The next day, carefully remove the top piece of paper and the cap.

4. Draw the spore print you see.

With forceps, remove some of the spores from the paper. Place them in a drop of wetting agent on a clean slide, and cover with a coverslip. Examine the spores under high power with the compound microscope.

5. Draw several spores. Note their color and estimate their size.

6. How does the mushroom spore compare in size, color, and shape with the spores of the black bread mold?

C. Yeast

Yeast is a common unicellular fungus. It usually reproduces by a process called budding. In budding, the cell divides, producing a very small cell that is attached to the larger parent cell. The small cell looks like a bud on the large cell. Eventually, the bud separates from the parent cell and grows on its own.

Place a drop of yeast culture on a clean slide and cover with a coverslip. Observe under high power with the compound microscope. Locate some yeast cells with buds. The buds will probably be of varying sizes.

7. Draw one of the budding yeast cells you see under the microscope. Estimate its size. Label the dividing nucleus (or two nuclei if budding is complete).

ANALYSIS

8. What are hyphae?

9. What part of a mold is colored?

10. What is a fruiting body of a mushroom?

11. Compare reproduction in the three types of fungi you observed in this lab. What similarities do you find in structure and in method of reproduction?

12. Bread mold, mushrooms, and yeast are quite different in appearance. What major characteristics cause them to be classified in the same kingdom?

FOLLOW-UP

Observe one or several yeast cells for a few minutes to learn whether you can see budding from start to finish. Try to determine at which point the single nucleus of the parent cell becomes two nuclei.

11 Science and the Scientific Method

PURPOSE

To become familiar with the means by which scientific information is gathered.

MATERIALS

paper

pencil

INTRODUCTION

Science is more than a body of information. Science is dynamic—it constantly grows and changes. Scientists carry on an unending search for new information. With that new information, they reevaluate old information to find out whether it is still valid.

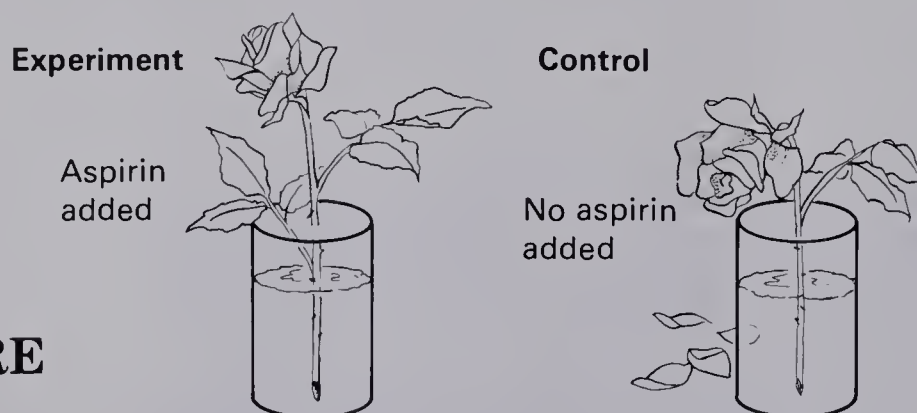
The scientific method offers a means of testing ideas and solving problems. Experimentation is the foundation upon which science rests, and experimentation is the heart of the scientific method. This lab gives you the opportunity to develop skill in using the scientific method.

In order to understand how to set up experiments, you must first become familiar with the following terminology:

- *Assumption* — a statement accepted without proof.
- *Hypothesis* — an idea used as the basis for experimentation. A hypothesis is expressed as a positive statement and is usually only one sentence long.
- *Deduction* — a statement that logically follows the hypothesis. If the hypothesis is correct, then such and such should happen. The “such and such” is the deduction. A deduction normally determines how an experiment will be designed.
- *Controlled Experiment* — the procedure designed to determine whether an idea—the hypothesis—is true or false.
- *Experiment* — the test in the controlled experiment. The experiment tests only one factor, as stated in the hypothesis.

- *Experimental Variable* — the single factor that the experimenter tests.
- *Control* — the standard of comparison for the experiment. All factors in the control are the same as in the experiment, except for the one factor tested in the experiment.
- *Fact* — something that is observed.
- *Data* — the facts collected during the experiment.
- *Analysis* — the interpretation of the data, or what the data mean.
- *Conclusion* — whether the hypothesis has been supported by the data. The conclusion of a controlled experiment is one of the following: The data support the hypothesis, or
The data do not support the hypothesis.

Hypothesis: Adding aspirin to water in a vase will extend the time that a cut rose stays fresh.



PROCEDURE

A. Applying the Terms of Science

The following fictional experiment demonstrates the use of the scientific method.

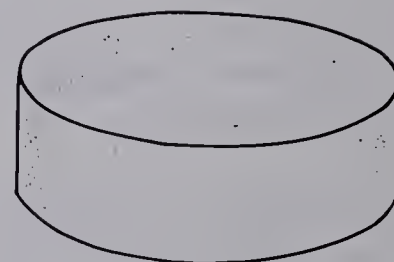
Many people in the small Midwestern town of Hootville are stricken with the disease "Buggo." Most of those stricken recover within seven to nine days.

Buggo has been shown to be caused by a bacterium called "Gotcha." Antibiotic "X" is a new drug that has been shown to kill Gotcha bacteria cultured in a test tube. Antibiotic X was also found to cure dogs that were stricken with Buggo.

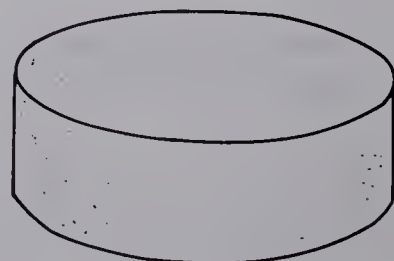
Researchers decided to test the new drug on some of the people in Hootville. They hypothesized that the drug would effectively cure Buggo in humans. They deduced that if they gave patients antibiotic X, the patients would recover more quickly than those who did not take the antibiotic.

The researchers prepared tablets containing antibiotic X. They also prepared a second batch of tablets that did not have antibiotic X. A tablet that does not contain any medication is called a placebo.

The researchers selected two groups of people who had just contracted the disease. Twenty-five people in group A were given the tablets containing the drug. Twenty-five people in group B were given the placebos.



**Tablet Containing
Antibiotic X**



Placebo—No Medication

Twenty of the twenty-five people given the drug recovered within one day. The other five people in group A took from seven to nine days to recover—the normal recovery time for untreated patients.

One of the people given the placebo recovered within one day. The rest of the people in group B took from seven to nine days to recover.

1. Name three facts that were known before the start of the experiment.

2. One assumption is, "Antibiotic X is not lethal to humans." List two other assumptions.

3. State the researchers' hypothesis.

4. One deduction is, "If people with Buggo are given a tablet containing X, they will be cured." List one other deduction.

5. How do the experiment and the control differ in this controlled experiment?

6. What are the data in this experiment?

7. What analysis do you think the researchers made of the data?

8. What conclusion do you think they reached?

B. Designing Your Own Experiment

Now it is your turn. Design an experiment to test the effect of fertilizer on bean production in bean plants. The story is:

Bean growers want to increase crop yields to meet the growing demand for food. The Acme Fertilizer Company has asked a botanist—you—to test their latest product, Growmore, on bean production.

9. Design an experiment to test Growmore.

Name _____ Date _____

10. What assumptions did you make?

11. What is the hypothesis for your experiment?

12. What deductions did you make?

13. What is your experimental control?

14. What would the data look like if they supported the hypothesis?

ANALYSIS

15. In a controlled experiment, why is there only one experimental variable?

16. What would happen if there were more than one variable?

17. Why is a control necessary?

18. What does a controlled experiment provide to help you solve a problem?

19. In the Buggo case, why did the researchers first test antibiotic X on a small group, instead of giving it to everyone in town who had the disease?

FOLLOW-UP

Can you think of a situation in your everyday life in which you could use the scientific method to solve a problem? For example, you might have a house plant that is not growing. You could test the effect of more or less water, light, or fertilizer on the plant.

Define the problem, make your assumptions, form a hypothesis, and test it in a controlled experiment.

12 Cell Structure

PURPOSE

To examine the structure of cells.

MATERIALS

methylene blue stain	flat-edge toothpick
cork	compound microscope
onion	slides and coverslips
single-edge razor blade	dissecting probe

INTRODUCTION

The cell is the basic unit of all living things. All organisms are made up of at least one cell. Large organisms, such as humans, are made up of trillions of cells. In this lab you are going to examine under the microscope three different kinds of cells: cork cells, onion cells, and your own cheek cells.

PROCEDURE

A. Cork Cells

Cork cells hold a special place in the history of biology. It was cork cells that Robert Hooke was looking at in 1665 when he coined the name "cells."

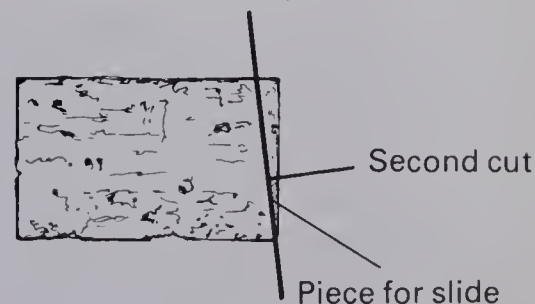
Commercial cork is obtained from the cork oak tree, which is native to the Mediterranean area. The outer bark of the tree produces these cells. This bark may be removed without damaging the tree.

To examine cork cells, you will need to cut a thin slice from a piece of cork. With a single-edge razor blade, cut a thin wedge from the end of the cork. Then, cut off the thick piece of the wedge. The remaining piece should be thin enough for light to pass through. If you find it difficult to cut a very thin piece, scrape the cork with the sharp edge of the razor blade. The small dustlike pieces should be suitable for viewing.

Place the cork tissue on a slide with a drop of water. Carefully position a coverslip on the slide so that the cork is in the center of the coverslip. Examine the slide under the compound microscope. Look for the boxlike structures that Hooke called cells. Note the thickened cell walls of the cork cells.

Caution: Cut the cork on a hard surface. Always cut in a direction away from your body.

First cut, as thin as possible



1. Draw several cork cells and estimate their size. Show the thickness of the cell walls. Label the cell walls.

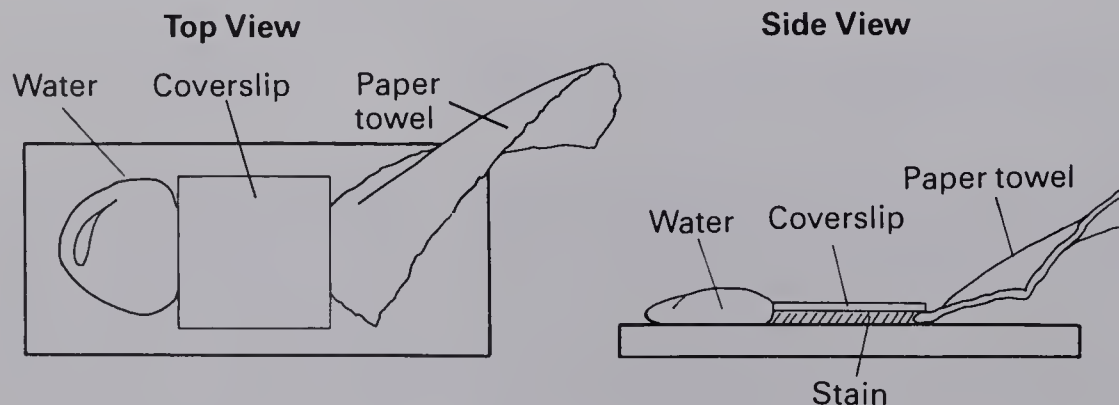
B. Onion Cells

The bulb of an onion is really an underground stem. The stem is completely covered by leaves, which take the form of succulent (full of juice) scales.

Obtain a piece of scale of an onion bulb. You will use the outer layer (the epidermal cells) from the scale. Bend the scale until it cracks, then gently pull the two pieces apart; the outer layer of epidermal tissue should peel off easily. This tissue will be about as thin and flexible as plastic wrap.

Now put a drop of water in the center of a clean slide. Cut off a small piece of the epidermal tissue and place it in the drop of water. Make sure that the tissue is flat. If it is folded, straighten it with a dissecting probe or needle. Put one drop of methylene blue stain directly on top of the onion tissue. Wait one minute, then place a coverslip over the tissue.

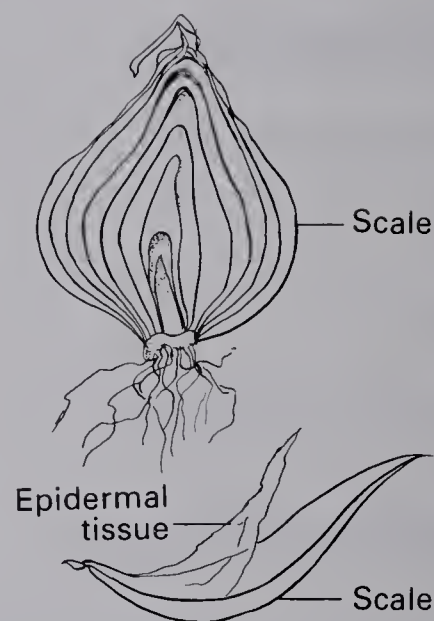
Next, remove the stain from under the coverslip and replace it with clear water. To do this, place a paper towel at the edge of one side of the coverslip. Place a drop of water at the edge of the coverslip on the other side.



The stained water under the coverslip will be absorbed by the paper towel. As the stain is removed, the clear water next to the coverslip will be drawn under the coverslip to replace the stained water.

After the stain is replaced with clear water, you will see that certain portions of the cell absorbed the stain well, while others did not. The stained parts of the cell are more visible under the microscope.

Observe the slide under the microscope. Try to identify the parts of the onion cell. Look for the nucleus, cytoplasm, and cell wall.



Take Care: Be very careful when using stains. Most stains are difficult to remove from skin and clothing.

2. How are the onion cells different from the cork cells?

3. Draw several onion cells and estimate their size. Label the cell wall, nucleus, and cytoplasm.

C. Human Cheek Cells

You will now observe some of your own cells. The epithelial cells lining your mouth are constantly being replaced. The old cells that are ready to slough off can easily be collected.

Put a drop of water on a clean slide. Using the flat end of a toothpick, gently scrape the inside of your cheek. Stir the water on the slide with the end of the toothpick to mix the cells with the water. If this does not produce any cells, scrape around your teeth at the gum-line. Epithelial cells that have sloughed off the cheek often mix in the saliva and get lodged in the teeth.

Apply one drop of methylene blue stain to the cells. Wait one minute and place a coverslip on the slide. Clear the slide of stain, using the technique described in part B.

Observe the slide under the microscope. Try to identify the parts of the cheek cell. Look for the cell membrane, the nucleus, and the cytoplasm.

4. Draw several cheek epithelial cells and estimate their size. Label the cell membrane, the nucleus, and the cytoplasm.

Take Care: If you have eaten soon before doing this part of the lab, you should rinse your mouth with water.

ANALYSIS

5. Would you describe the shape of the cheek cells as regular (all the same shape) or irregular?

6. Would you describe the onion cells as regular or irregular?

7. Would you describe the cork cells as regular or irregular?

8. Do you find a cell membrane or a cell wall at the outer edge of a plant cell such as an onion cell?

9. Do you find a cell membrane or cell wall at the outer edge of an animal cell such as a human cheek cell?

10. A cell wall is not living, while a cell membrane is a living structure. Write a hypothesis based upon this information to account for the regularity or irregularity you found in the shapes of living cells.

11. What structures did methylene blue stain in the plant tissue? in the animal tissue?

13 Measuring pH

PURPOSE

To learn what acids and bases are and how they are measured on the pH scale.

MATERIALS

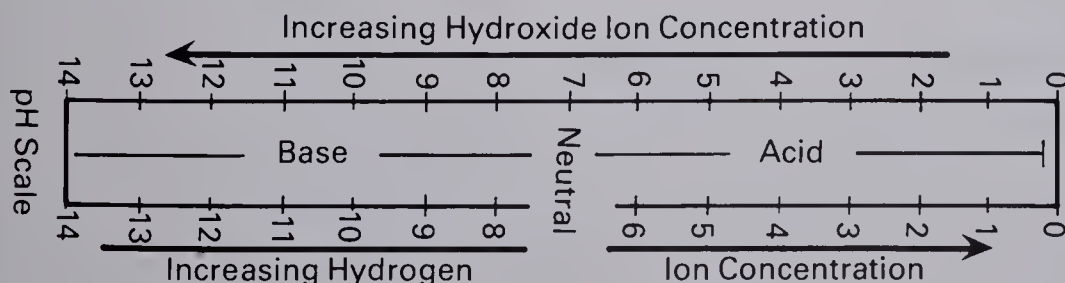
antacid tablet dissolved in water	water
carbonated beverage	0.1 M hydrochloric acid
egg white	0.1 M sodium hydroxide
lemon juice	color chart
piece of raw potato crushed in water	forceps
saliva	pH paper
tomato juice	spot plate

INTRODUCTION

Many substances are classified according to the chemical quality of being acidic or basic. To measure this quality, scientists use the pH scale.

The pH scale measures how acidic a solution is. The scale ranges from 0 to 14, and the meaning of these values is the opposite of what you might expect. The lower the pH value, the stronger the acid is. A solution with a pH of less than 7 is classified as an acid.

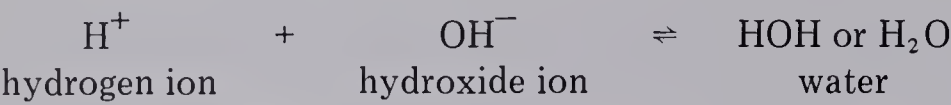
The higher the pH value, the weaker the acid is. A solution with a pH above 7 is low in acidity and is classified as a base. So, while the pH scale measures acidity, it also measures how basic a solution is.



A pH value of 7—the midpoint of the scale—indicates a neutral solution. A neutral solution is neither acidic nor basic.

Each consecutive number on the pH scale represents a tenfold change in acidity or basicity. For example, a solution with a pH of 5 is ten times more acidic than a solution with a pH of 6.

In water solutions, whether a solution is acidic or basic depends on the relative concentration of hydrogen ions (H^+) and hydroxide ions (OH^-). Acidic water solutions have more H^+ ions than OH^- ions. Basic water solutions have more OH^- ions than H^+ ions. Pure water, which is neutral, contains equal numbers of H^+ ions and OH^- ions.



Acids add H^+ ions to water solutions. For example, hydrochloric acid (HCL), which is a strong acid, separates into H^+ ions and Cl^- ions in water. This raises the number of H^+ ions in the solution. Bases combine with H^+ ions in water solutions. For example, sodium hydroxide (NaOH), which is a strong base, separates into Na^+ and OH^- in water. The OH^- ions combine with H^+ ions in the water. This lowers the number of H^+ ions in the solution.

Many biological reactions require specific pH ranges in order to function properly. The pH of solutions inside and outside of cells greatly influence chemical processes. Digestive enzymes in the mouth work best at a pH of 6.8, and those in the stomach work best at a pH of 2. Stomach enzymes do not work at a pH of 9.

In this lab you will test several substances to find their pHs.

PROCEDURE

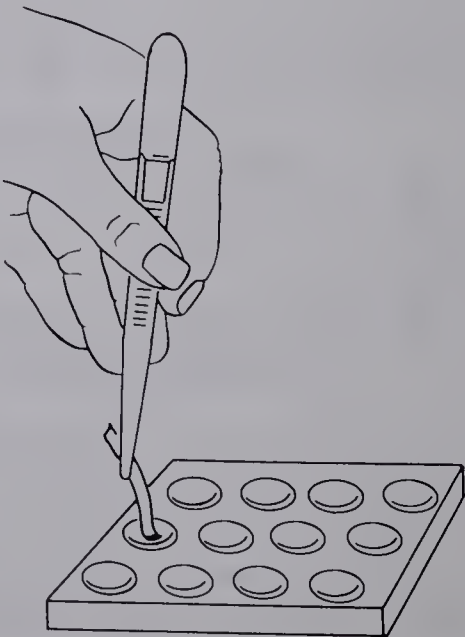
The pH of a solution is tested with special dyes. These dyes change color as the number of H^+ ions in the solution changes. The dyes therefore indicate the number of H^+ ions in a solution.

The dyes are usually put on a strip of paper called pH paper. The pH paper is keyed to a color chart, which shows the color that the paper will be at different pH values.

Put a few drops of each solution to be tested on a spot plate. You will need about 1 cm of pH paper to test each solution. Holding the paper with forceps, dip one end of the paper into the solution. Avoid touching the paper with your hands. Match the color of the pH paper to the color chart to determine the pH of the solution. Discard the used pH paper, and record your pH data on the data chart. Repeat this procedure for each solution, using a new piece of pH paper every time.

Take Care: pH paper is expensive and should be used sparingly. Also, keep the color chart clean and dry. Without a legible color chart, pH paper is of little value.

Substance	pH value	Substance	pH value



Next, place three drops of sodium hydroxide in a clean depression on your spot plate. Add three drops of hydrochloric acid to another spot. Test the pH of each.

Now, place three drops of hydrochloric acid and three drops of sodium hydroxide in the same spot. Allow them to mix for one minute. Test the pH.

Caution: Hydrochloric acid and sodium hydroxide are strong chemicals that can burn skin and clothing. Handle them with care.

1. What is the pH of hydrochloric acid? of sodium hydroxide?

2. What is the pH of the sodium hydroxide and hydrochloric acid mixture?

ANALYSIS

3. How do you explain the change of pH when you mix an acid with a base?

4. When you mix an acid and a base, you neutralize the solution. Why do you think this term is used?

5. List the materials you tested in order from the most acidic to the most basic.

6. Which has more hydrogen ions, a solution with a pH of 10 or a pH of 8?

7. Which has more hydroxide ions, a solution with a pH of 10 or a pH of 8?

8. What is the pH value of a neutral solution?

9. Why is the pH of solutions in the body important?

14 Enzyme Function

PURPOSE

To explore the role of enzymes in chemical reactions.

MATERIALS

chalk	manganese dioxide
potato	boiling water
raw hamburger	graduated cylinder
raw liver	scalpel
boiled liver	10-mL test tubes
spinach leaf	test tube rack
3 percent hydrogen peroxide	

INTRODUCTION

Enzymes are some of the most important kinds of molecules found in living cells. Cells could not function without enzymes. They control the chemical reactions of the cells.

To understand how enzymes work, you will be observing some simple chemical reactions with hydrogen peroxide. You may have hydrogen peroxide in your medicine cabinet at home. It is commonly used as a bleaching and disinfecting agent.

Hydrogen peroxide has the chemical formula H_2O_2 . Notice the similarity between this formula and the formula for water, H_2O . Hydrogen peroxide is an unstable compound, which turns into water by the following reaction:



Hydrogen peroxide is stored in a brown bottle to keep out light, which speeds up this reaction. Certain chemicals can also dramatically increase the speed at which this reaction takes place. In this lab you will work with some of them.

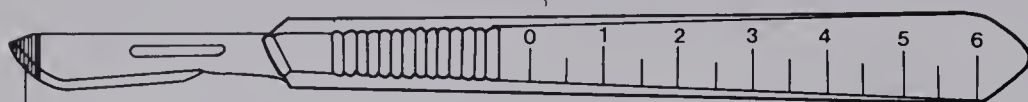
PROCEDURE

Measure 2 mL of hydrogen peroxide in a graduated cylinder and pour

Caution: Be careful not to get the hydrogen peroxide on your skin or your clothes. It is a powerful bleach.

it into a test tube. Add to the test tube enough manganese dioxide, MnO_2 , to cover the end of a scalpel (as illustrated).

The shaded end of the scalpel is used to measure.



Substance (manganese dioxide, chalk, hamburger, liver, potato, spinach) should cover end of scalpel.

1. Describe the reaction.

2. When the reaction ends, add 2 mL more hydrogen peroxide to the test tube. Does the reaction occur again?

3. When the reaction ends, add a little more manganese dioxide. Does the reaction occur again?

4. Based on your observations, which of the following statements is the most probable assumption? Circle the letter of the statement you choose.

- a. The hydrogen peroxide is used up and the manganese dioxide remains unchanged.
- b. The manganese dioxide is used up and the hydrogen peroxide remains unchanged.
- c. Both the hydrogen peroxide and the manganese dioxide are used up.
- d. Neither the hydrogen peroxide nor the manganese dioxide is used up.

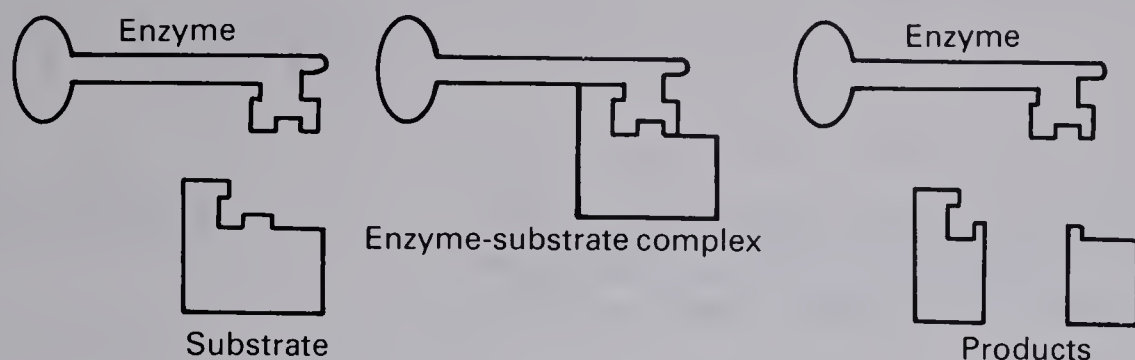
In this reaction, manganese dioxide was a catalyst. A catalyst is a substance that speeds up a reaction but remains unchanged by the reaction.

In living organisms, enzymes act as catalysts. All enzymes are made of protein. Therefore, enzymes are often described as protein catalysts.

For each type of chemical reaction that occurs in the cell there is a specific enzyme. Just as a key fits only one lock, an enzyme works in only one reaction. The chemicals upon which the enzyme acts in the reaction are called substrates.

Biologists usually give enzymes names that end in *-ase*. The first part of the enzyme name comes from the substrate upon which the enzyme acts. For example, the enzyme that breaks down the substrate sucrose is called sucrase.

This naming system is useful to remember, but unfortunately it does not always hold true. The enzyme in living organisms that acts upon



the substrate hydrogen peroxide may properly be called hydrogen peroxidase. However, it is usually called catalase.

Hydrogen peroxide in plants and animals occurs as a by-product of cellular respiration, the process by which cells obtain energy from food. Water is usually formed at the end of this process. However, hydrogen peroxide is sometimes formed instead. When this happens, catalase immediately breaks down the hydrogen peroxide into oxygen and water.

To demonstrate the action of catalase from foodstuffs on hydrogen peroxide, perform the following tests.

Pour 2 mL of hydrogen peroxide into each of five test tubes. With the scalpel, chop enough chalk, hamburger, liver, spinach, and potato to cover the end of the scalpel. Be sure to prepare the same amount of each item and to keep the items separate. Place each in a tube with the hydrogen peroxide.

Note the speeds of the reactions. The faster the reaction, the more vigorously the liquid bubbles, and the warmer the test tube gets. On the data chart, list the items in order from the fastest reacting to the slowest reacting.

FOODSTUFFS

<i>fast reacting</i>	
<i>slow reacting</i>	

5. Which substances contain more of the enzyme, those at the top of the list or those at the bottom?

6. Are the meats near the top or the bottom of the list? the plants?

7. Which, if any, of the items did not produce a reaction? If a substance produced no reaction, explain why.

Chop a piece of boiled liver large enough to cover the end of the scalpel. Chop an equal amount of fresh liver. Pour 2 mL of hydrogen peroxide into two clean test tubes. Add the boiled liver to one tube and the fresh liver to the second tube.

8. Describe what happens.

9. Based on this test, what assumption can you make about the effect of boiling enzymes?

ANALYSIS

10. What two products does hydrogen peroxide change into when it breaks down?

11. What effect does a catalyst have on this chemical reaction?

12. What do you think causes the bubbling in this reaction?

13. What is the function of an enzyme?

14. What enzyme acts on hydrogen peroxide in living organisms?

15. Would this enzyme act on chemicals other than hydrogen peroxide? Why or why not?

15 Leaves

PURPOSE

To study the general structure of leaves and to contrast monocot and dicot leaves.

MATERIALS

ivy leaves	dissecting needle
<i>Zebrina</i> leaves	forceps
prepared slide of maple leaf (or linden) cross section	compound microscope
cold water	slides and coverslips

INTRODUCTION

The leaf is the primary photosynthetic organ of the plant. Each part of the leaf plays a role in photosynthesis. Some parts supply the necessary materials, and other parts, which contain chlorophyll, carry out photosynthesis.

Although all leaves perform the same major function, leaves vary widely in shape and structure. In this lab, you will observe two major types of leaves and become familiar with general leaf structure.

PROCEDURE

A. Cross Section of a Leaf

All leaves are made up of three kinds of tissue: epidermis, mesophyll, and veins. Each tissue plays a different role in photosynthesis.

The epidermis forms the outer, protective layer of the leaf. It is a single cell in thickness. Covering the epidermis is the waxy cuticle. The cuticle limits water loss to the atmosphere through evaporation and protects the leaf from injury. The cuticle covers the top and bottom surfaces of the leaf and is usually thicker on the top surface.

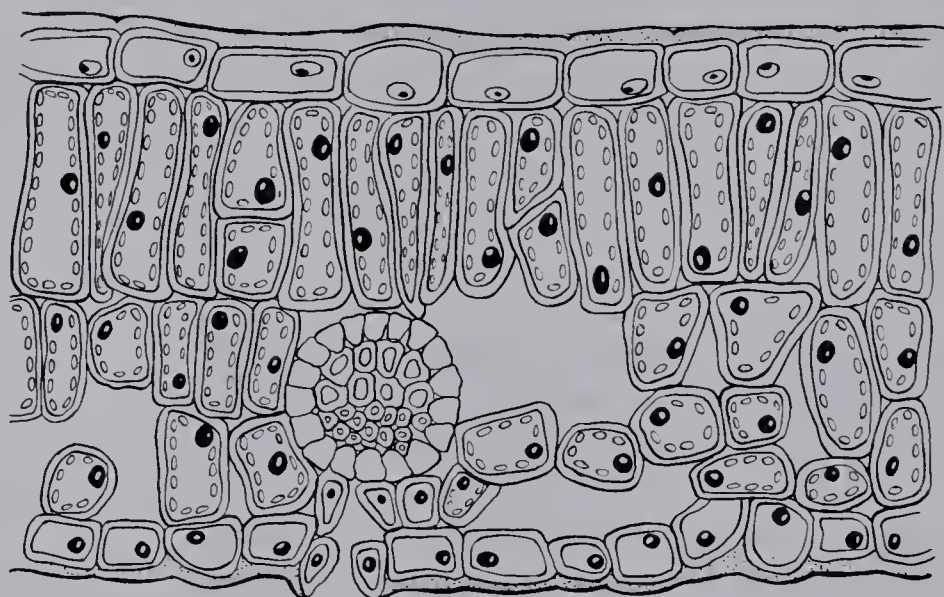
The epidermis also contains pores called stomates. Stomates may be found on both sides of the leaf, but are usually more numerous in the lower epidermis on the underside. Stomates allow gas exchange between the leaf and the atmosphere. The stomates are surrounded by bean-shaped guard cells, which open and close the stomates. The guard cells are the only cells in the epidermal layer that contain chloroplasts and perform photosynthesis.

Mesophyll, the second kind of leaf tissue, contains chloroplasts and is the primary site of photosynthesis. It is usually constructed of two

layers. The palisade layer, beneath the upper epidermis, consists of column-shaped cells. Below this is the spongy layer, which has loosely packed cells and many air spaces. The air spaces are connected to the stomates and aid in gas exchange. Air moves through the air spaces to all parts of the leaf.

Running through the mesophyll is the third kind of leaf tissue, veins. They conduct materials to and from the leaf and help support the leaf. Veins are bundles of tubes that are connected to the vascular system of the stem. Each bundle contains xylem and phloem. Xylem transports water and minerals from the roots and stem to the leaf. Phloem transports food manufactured by photosynthesis in the leaf to other parts of the plant.

1. On the leaf cross section shown, identify the parts just described. Label the cuticle, upper epidermis, chloroplasts, mesophyll, palisade layer, spongy layer, air space, vein, lower epidermis, stomate, and guard cells. Indicate which cells contain chloroplasts.



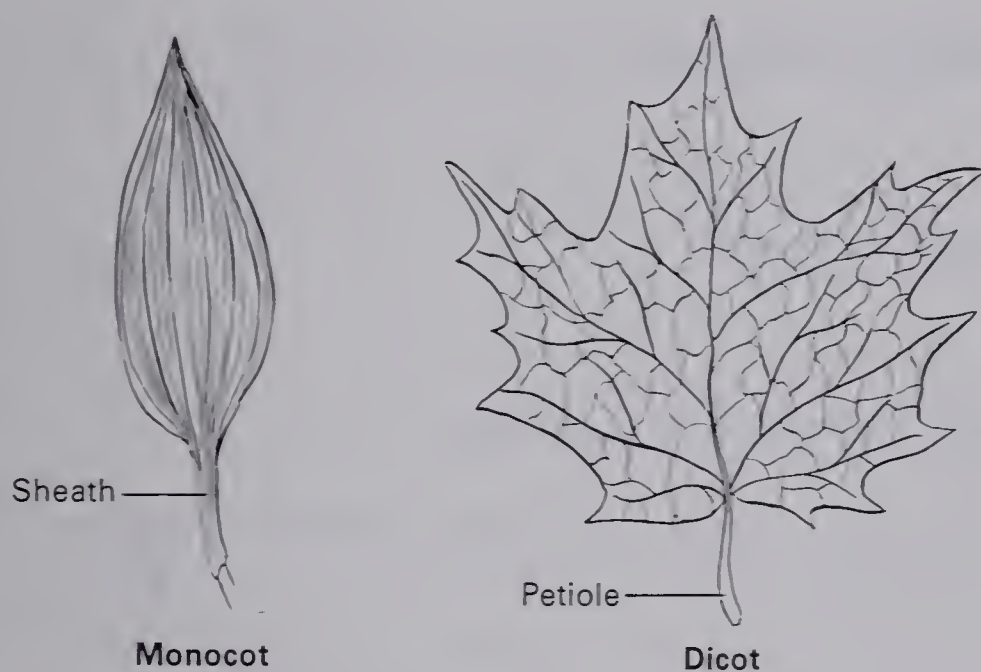
Examine the prepared slide of a maple leaf cross section under the compound microscope. Look for the parts that you labeled on the diagram.

2. Draw a small portion of the leaf cross section as it appears under the microscope and label the parts.

B. Monocot and Dicot Leaves

General Structure The leaves of all flowering plants have certain characteristics, depending on whether the plant is a monocot or a dicot. The major difference between the two plant groups is the number of cotyledons (the seed leaves) in the seed. A monocot has a single cotyledon and a dicot has two.

The leaves of monocots and dicots differ in several ways. In most dicots, the broad part of the leaf, called the blade, is connected to a stalklike petiole. The petiole attaches the leaf to the branch. In most monocots, the blade extends into a sheath, which wraps around the stem.



The vein patterns are also different in monocots and dicots. Most monocots have many veins of similar size that are parallel to each other. Most dicots have a large vein in the middle of the blade, from which smaller veins branch out. This pattern is called netted venation.

To observe these differences, examine the ivy leaf and the *Zebrina* leaf.

3. Which leaf has a petiole? Which has a sheath?

4. Contrast the vein patterns of the two leaves.

5. Which plant is the monocot and which is the dicot?

Epidermis Monocot and dicot leaves have the same tissues—epidermis, mesophyll, and veins—but the tissues may show variations. In the epidermis of a monocot, stomates occur in rows that are parallel to the long axis of the leaf. Dicot stomates are randomly arranged.

To see the stomate arrangement, you will need to peel off the epidermal layer of the ivy leaf and the *Zebrina* leaf. Obtain an ivy leaf that has been soaking in cold water. Bend the leaf until it cracks, but do not tear it all the way through. Use a forceps to peel off a piece of the thin, colorless lower epidermis. Put the lower epidermis tissue on a clean slide and straighten any wrinkles with a dissecting needle. Add a drop of water to the tissue and cover with a coverslip. Follow the same procedure to make a wet mount of the *Zebrina* leaf.

Observe the slides under the compound microscope. Look for the stomates. If they are open, they will appear as colorless holes. If the stomates are closed, you will have to locate them by finding the green bean-shaped guard cells.



6. In which leaf are the stomates arranged in parallel rows?

7. In which leaf are the stomates randomly arranged?

8. Which leaf is the monocot and which is the dicot? Is this consistent with your answer to question 5?

9. Draw a stomate with its guard cells, as it appears on the ivy and on the *Zebrina*.

ANALYSIS

10. What is the primary function of all leaves?

11. Which parts of the leaf perform photosynthesis? How can you tell?

12. Which two parts of the leaf are directly involved with the conduction of gases in the leaf?

13. How does the leaf get the carbon dioxide, water, and minerals it needs to perform photosynthesis?

14. List three differences between most monocot and dicot leaves.

15. The cuticle of a leaf is usually thicker on the top side than on the bottom side. How does this adaptation benefit the leaf?

FOLLOW-UP

Make a brief survey of the plants around your school or a nearby park.
Are most of the leaf types monocot or dicot?

16 Separating Plant Pigments by Chromatography

PURPOSE

To discover which pigments are in a plant by using paper chromatography.

MATERIALS

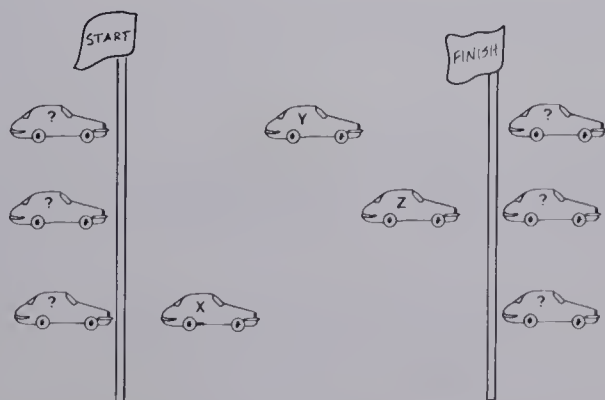
ethyl alcohol	hot plate
package of frozen spinach	tongs or hot pads
petroleum ether-acetone solvent	test tube rack
150 mL beaker	two test tubes
capillary tube	cork stoppers to fit tubes
chromatography paper	paper clip
	pencil

INTRODUCTION

Pigments, located in the cells and tissues of organisms, produce the appearance of color. Pigments are simply very large molecules that reflect light. One way to find out which pigments are in a plant is to use a technique called paper chromatography. This separates the different molecules.

Suppose that you are at an automobile speed race among three cars. You know that car X can travel 80 mph, car Y can go 95 mph, and car Z can go 110 mph. However, you do not know which car is which. So, when the three cars are lined up at the starting position, you cannot tell which car is fastest.

Shortly after the race begins, you can figure out which car is which. As car Z reaches the finish line, the three cars are the maximum dis-



tance apart. Shortly thereafter, cars Y and X will also cross the finish line. When this happens, it will again be impossible to tell the cars apart.

The same thing happens to molecules on a paper chromatogram.

The molecules are placed at one end of a strip of paper. Then, the end of the paper is placed into a solvent. The solvent is absorbed and travels up the paper. As the solvent passes the molecules, it carries them along. Different molecules are carried at different rates along the paper by the solvent. Larger molecules travel more slowly than smaller molecules.

Eventually the solvent reaches the other end of the paper strip. Just as all the cars in a race eventually cross the finish line, all the molecules carried by the solvent will get to the other end of the paper strip. If you wish to separate the different molecules, you must stop the race before they all reach the finish line.

PROCEDURE

A. Extracting Pigment From Leaves

Several groups of students can work together to extract the pigment from spinach leaves. Remove as much excess water as possible from thawed spinach leaves by pressing them between paper towels. Fill a beaker half full with the leaves. Pour enough ethyl alcohol into the beaker to cover the leaves completely. Boil the alcohol over a hot plate until the liquid becomes dark green.

As the liquid boils it removes the pigments from the spinach leaves, and the alcohol becomes a darker and darker green. The more concentrated the pigment solution is, the better the chromatogram will be.

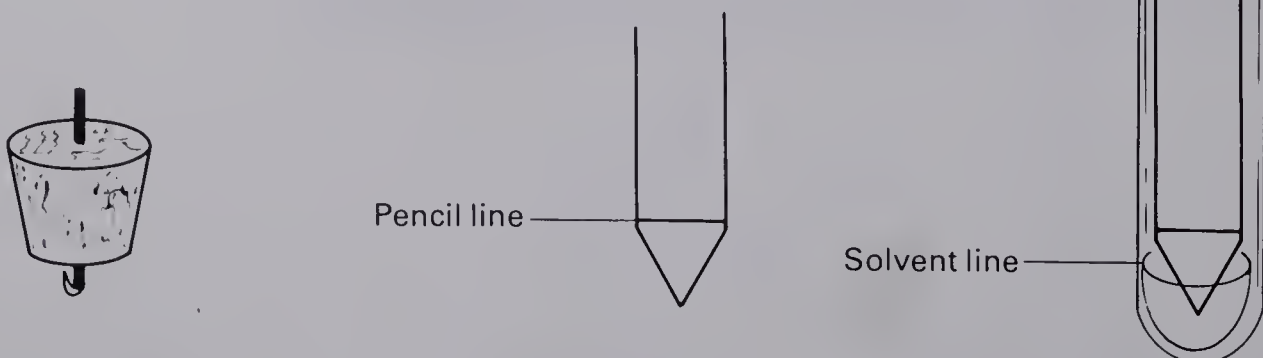
Remove the beaker from the hot plate and allow the liquid to cool. Each group should pour off 1-2 mL of the dark green liquid into a test tube. Do not let any of the leaf debris get into the tube. This liquid will be used for making the paper chromatogram.

Caution: Do not use an open flame anywhere near the boiling alcohol. When handling the heated beaker, be sure to use tongs or hot pads to protect your hands.

B. Separating the Pigments

Obtain a test tube with a tight-fitting cork. Fashion a hook out of a paper clip and push it through the cork as shown.

Cut a strip of chromatography paper slightly shorter and narrower than the inside of the test tube. The paper should easily slide in and out of the tube without rubbing the sides. With a pencil, *not a pen*, draw a line 1 cm from one end of the paper strip. Cut a point on that end of the paper.



The paper should fit inside the tube as shown. Mark the outside of the test tube to show the proper solvent level. The tip of the paper should be below the solvent line, but the pencil line on the paper should be above the solvent. Remove the paper and lay it aside.

Pour the solvent into the tube up to the marked line. Replace the cork (without the paper). This will saturate the air in the tube with solvent. Leave the cork in the tube while you prepare the paper strip.

Dip a capillary tube into the alcohol pigment solution. Wipe the excess solution off the tube with a paper towel.

Place the next drop that comes out of the capillary tube onto the line on the paper strip. Let the spot dry completely. You may blow on the spot or wave the paper in the air to speed the drying. Add another drop, directly on top of the first. Try not to overlap the drops—the green spot should be as small as possible. Dry, and repeat the process about 15 times. Remember, the spot must be completely dry before each drop is added. Applying the drops to the paper will take at least five minutes. When you have finished the paper should have a very small, dark-green spot.

When the last drop is completely dry, remove the hooked cork from the tube, place the paper strip on the hook, and insert it into the tube. The spot *must* remain above the liquid solvent. Make any minor adjustments with the paper clip, if necessary.

Let the tube stand motionless in the test tube rack until the solvent moves up the paper to the hooked paper clip—about 15 minutes.

Immediately remove the paper from the tube. Make a pencil line marking the farthest point reached by the solvent. This line marks the solvent front. Let the paper dry.

Staple your chromatogram to this sheet in the space provided. If you are working with a partner, cut the strip in half lengthwise so that each of you has a chromatogram.

You should be able to distinguish several pigments. Chlorophyll A is yellow-green, and chlorophyll B is blue-green. You may also find some other pigments. The yellow-orange pigment is carotene and the yellow pigment is xanthophyll.

1. Label the pigments on your chromatogram. (See right.)

Different colors are sometimes hard to distinguish on a chromatogram. A more precise way to identify different pigments is to calculate the relative speed of movement. You must measure two distances: for the solvent and the pigment. The distance that the solvent moved is equal to the distance between the two pencil lines. The distance that the pigment moved is measured from the bottom pencil line to the uppermost edge of the pigment spot.

Now you can calculate the relative speed of movement, which is called the reference flow factor, R_f . Divide the distance the pigment moved by the distance the solvent moved to get the R_f .

$$R_f = \frac{\text{distance pigment moved}}{\text{distance solvent moved}}$$

Caution: Do not inhale the fumes from the solvent.

Take Care: Hold the paper by the edges. Fingerprints on the center of the paper will ruin the chromatogram.

Staple your chromatogram here.



The R_f is different for every pigment. Once you have identified the R_f of a pigment, you will find that it is the same in any paper chromatogram, as long as the same solvent is used.

ANALYSIS

2. Calculate the R_f value for chlorophyll A. Put your answer in the space below and on the chalkboard.

3. Determine the class average R_f value for chlorophyll A.

4. How does your R_f value for chlorophyll A compare with the class average?

5. Calculate the R_f values for the other pigments separated on your chromatogram.

6. Why is it important to remove the paper from the tube as soon as the solvent reaches the paper clip?

FOLLOW-UP

Using the technique you learned in this lab, make a chromatogram of pigments in other kinds of leaves. Devise a method for identifying the pigments based on your results from this lab. Are the pigments the same as or different from the spinach pigments? Why or why not?

17 Gas Production in Photosynthesis

PURPOSE

To determine the rate of gas production during photosynthesis and to determine the kind of gas produced.

MATERIALS

Part A, per team of 2-4:

- | | |
|---|--|
| 6 sprigs <i>Elodea</i> (<i>Anacharis</i>) | 3 pieces of flexible rubber tubing, 30 cm long |
| sodium bicarbonate | |
| boiled water | 3 disposable hypodermic syringes (1-2 mL) |
| 3 large test tubes | test tube rack or beaker |
| 3 one-holed soft rubber stoppers that fit tightly in test tubes | aluminum foil |
| | food coloring |
| 3 pieces of capillary tubing (15 cm long) | wax pencil |
| 3 pieces of fire-polished glass tubing (5 cm long) | 3 metric rulers |
| | tape |

Part B, per class:

- | | |
|--|---|
| 6-10 sprigs <i>Elodea</i> (<i>Anacharis</i>) | large clear glass funnel that fits beaker |
| boiled water | |
| large beaker | small test tube |
| | wooden splint |

INTRODUCTION

Photosynthesis is the process by which green plants and algae produce food. During photosynthesis, they use energy from light to convert water and carbon dioxide into glucose and oxygen. The oxygen that is produced is released into the atmosphere as a gas.

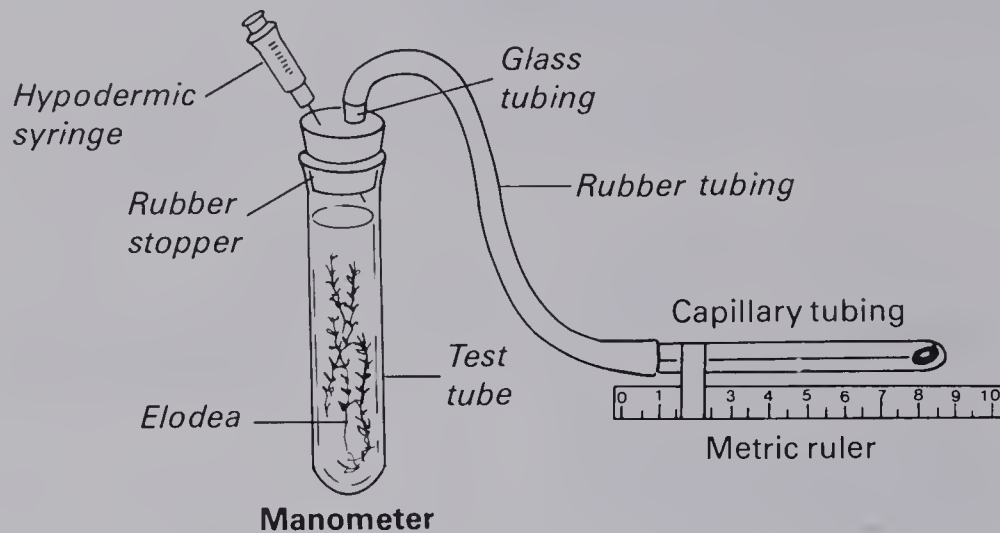
The rate at which plants produce gas can be determined by measuring the pressure of the gas produced. This measurement is made with an instrument called a manometer.

In this lab, you will use a manometer to determine the rate of gas production during photosynthesis. To verify that the gas produced is oxygen, you will perform a simple test.

PROCEDURE

A. Measuring the Rate of Gas Production

Each team will need three manometers, set up as illustrated.



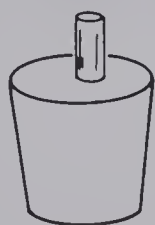
First, prepare each of the three rubber stoppers. Wet a 5-cm-long piece of glass tubing and gently insert it through the stopper from the top side. The bottom of the tube should be flush with the bottom of the stopper.

Next, fit a 30-cm-long piece of rubber tubing over the top end of the glass tubing. Fit the other end of the rubber tubing over a 15-cm-long piece of glass capillary tubing. Tape a metric ruler to the capillary tubing, as illustrated.

Finally, insert a hypodermic needle through the rubber stopper so that only the end of the needle comes through the bottom of the stopper.

Caution: Do not *force* the tube into the stopper—the tube may break. If you press hard enough on the tube to break it, the broken glass could be forced into your hand.

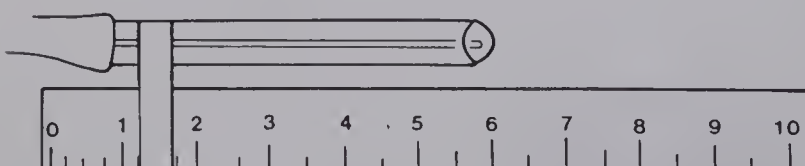
1. Insert glass tubing into stopper.



2. Attach rubber tubing.



3. Tape ruler to capillary tubing.



4. Insert needle through stopper.



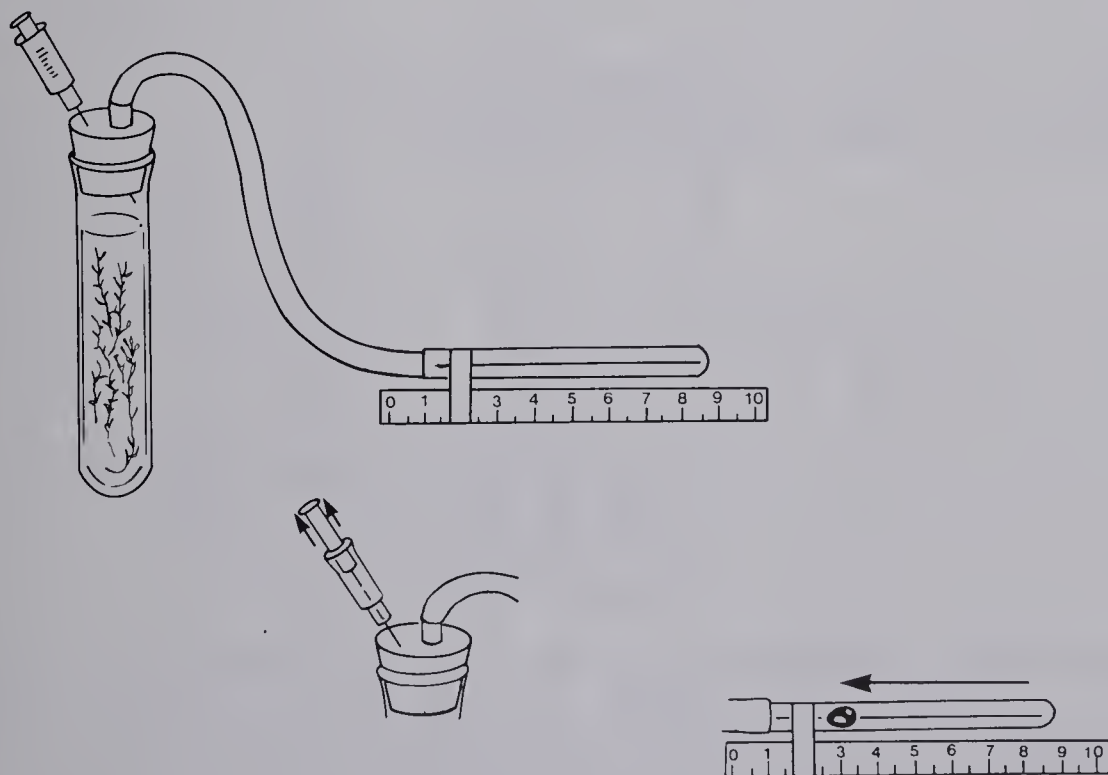
Label the three test tubes X, Y, and Z. Place three sprigs of *Elodea* in tubes X and Y. Push the plants far enough into the tubes to leave about 2 cm of space at the top.

Pour enough prepared water into tubes X and Y to cover the elodea, leaving an air space at the top. Add the same amount of prepared water to tube Z. The water has been boiled to remove all of the dissolved air. Sodium bicarbonate was added to the water to provide plenty of carbon dioxide for photosynthesis.

Place the prepared rubber stoppers in each of the three tubes. They should fit snugly. Check to be sure that there is an air space between the bottom of the stopper and the top of the water in each tube. If necessary, pour out some of the water.

Completely cover tube Y with aluminum foil, so that no light can reach the contents of the tube.

Place a drop of food coloring in the end of the capillary tube. Pull up on the syringe plunger to draw the drop into the capillary tube—it should be near the junction of the rubber tubing and the capillary tubing. The syringe plunger may be used to make periodic adjustments of the drop in the capillary tube. If the colored liquid approaches the end of the tube, return the drop to the starting position and continue taking readings.



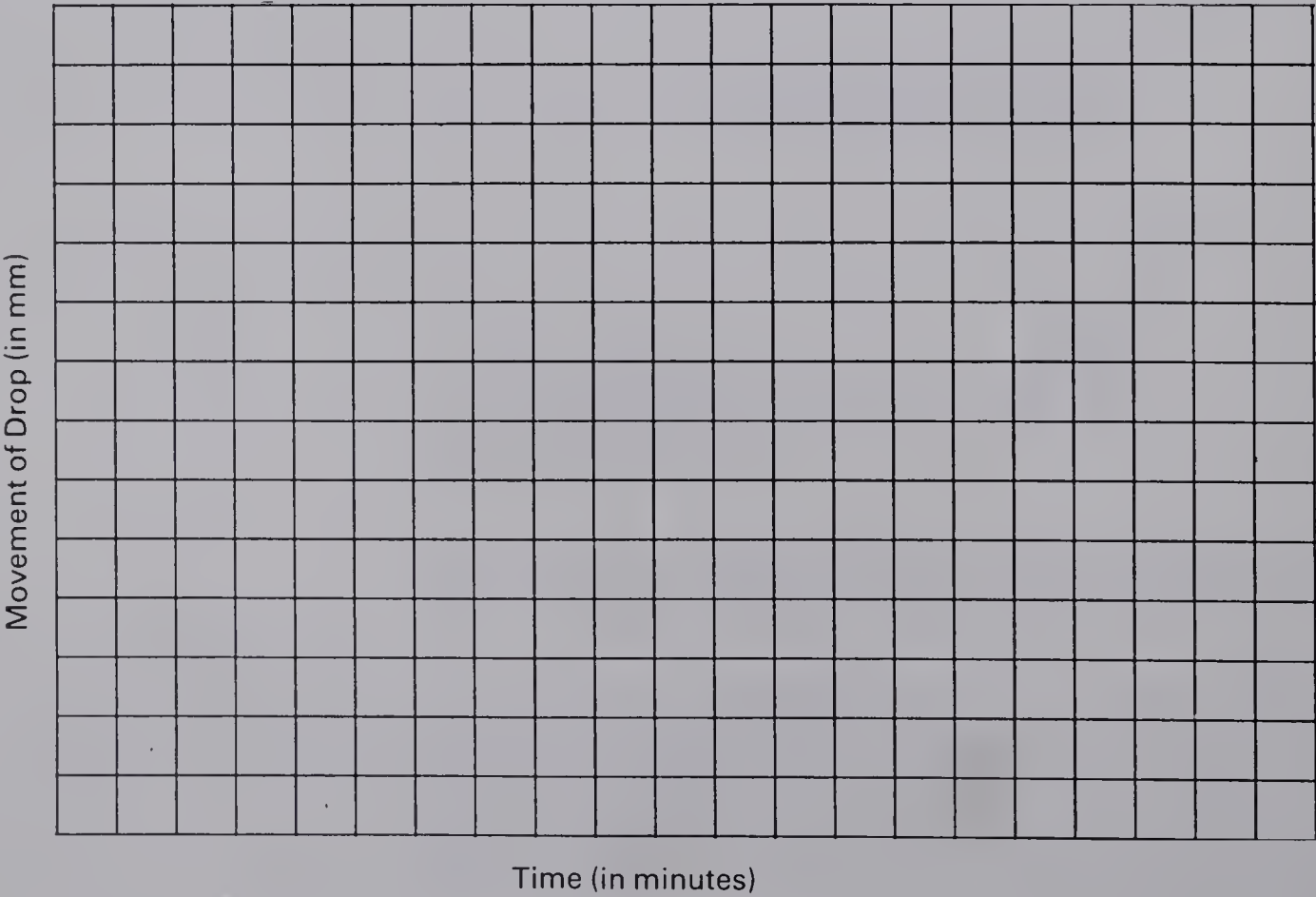
Place all three tubes in a test tube rack or a beaker. Let them stand in sunlight or, if that is not possible, in a strong artificial light for about one minute.

Note the position of the colored drops in relation to the rulers. As gas is produced in the tube, the pressure will increase and push the colored drop outward. Outward movement is considered positive (+) movement. If the amount of gas in the tube decreases, the pressure will decrease and pull the colored liquid inward. Inward movement is considered negative (–) movement.

On the data chart, record the change in the drop's movement every 2 minutes for at least 20 minutes (longer, if possible). For example, if your drop starts at 5 mm and moves to 8 mm, record "+3 mm." If at your next reading the srop goes from 8 mm to 12 mm, record "+4 mm" on the chart.

<i>Time (minutes)</i>	<i>Tube X</i>	<i>Tube Y</i>	<i>Tube Z</i>
0			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			

1. Make a graph of your data. For each tube, use a different color pencil or a different marking (for example, dashes, dotted lines, solid lines).



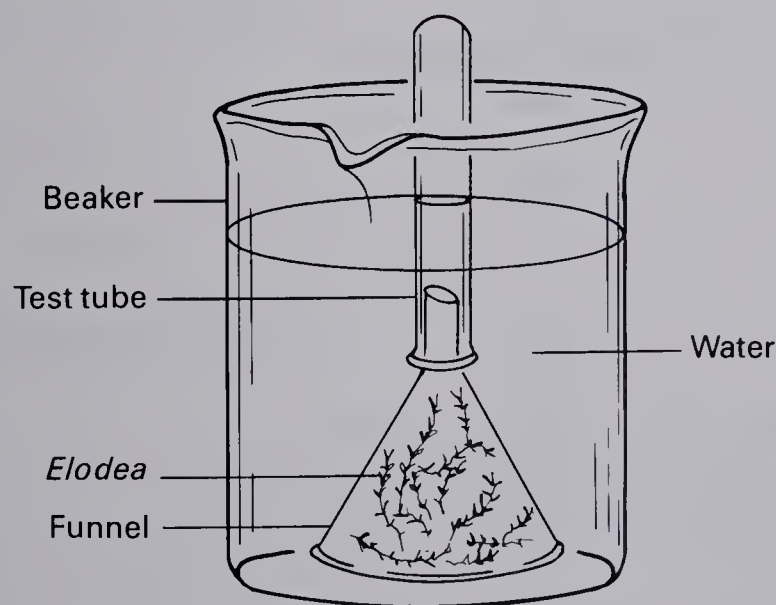
2. What was the purpose of tube Z without the plant?

3. What was the purpose of tube Y covered with foil?

4. Did the rate of gas production become relatively stable during the experiment?

B. Analysis of the Gas

Refer to the illustration shown while setting up the apparatus. Invert a large glass funnel inside a large beaker. Put as much fresh *Elodea* under the cone of the funnel as will fit.



Pour boiled water that has cooled to room temperature into the beaker. The neck of the funnel must be completely under water.

Fill a small test tube with the prepared water and cover the end with your thumb. Invert the tube in the beaker, holding your thumb so that none of the water comes out of the tube. Position the tube over the neck of the funnel and remove your thumb. The gas given off by the *Elodea* will be collected in the inverted tube and will slowly displace the water.

Place the beaker in sunlight or, if that is not possible, in a strong artificial light. It may take several days for the tube to fill with gas. Several factors determine the time: the health of the plants, temperature, and the amount and wavelength of the light.

When the water level in the tube is about halfway between top and bottom or lower, there is enough gas in the tube to test. The positive

test for oxygen is for a glowing splint inserted into the tube to burst into flame. Remove the gas-filled tube from the funnel and immediately cover it with a thumb.

Meanwhile, light a wooden splint. When it is burning well, blow out the flame. Quickly thrust the glowing splint into the tube.

Caution: Take great care when handling flammable materials.

5. Did the splint burst into flame, indicating that the gas is oxygen?

ANALYSIS

6. Based upon the results of the experiment, was light necessary for the plants to produce oxygen? What evidence supports your answer?

7. What raw materials does a plant use in photosynthesis, and what products does it make?

8. Where does a plant get energy to carry on photosynthesis?

9. Could a manometer be used to measure the pressure of gases other than oxygen?

FOLLOW-UP

Design and perform an experiment to determine the change in the rate of gas production with varying intensities of light or different colors of light. Use the same manometer setup that you used in this lab.

18 Nutrition

PURPOSE

To learn how to identify carbohydrates, lipids, and proteins in food-stuffs.

MATERIALS

corn and bean seeds	iodine indicator solution
gelatin	distilled water
potato	boiling water bath
vegetable oil	brown paper grocery bag
Benedict's solution	eyedropper
0.1 and 1.0 percent glucose solutions	10-mL graduated cylinder
starch solution	test tubes
14 M concentrated nitric acid	tongs

INTRODUCTION

You are what you eat. You have probably heard that statement many times, and there is some truth in it. Certain large organic molecules are necessary ingredients in the diets of humans and most animals.

We eat three groups of organic compounds: carbohydrates (starches and sugars), lipids (fats and oils), and proteins. Carbohydrates are used mainly as sources of energy. Certain lipids make up an important part of cellular membranes. Others are energy-storage molecules. Proteins form enzymes, the molecules that control chemical reactions in cells. Proteins are also important cellular components in many tissues, such as muscles and other body organs.

In this lab you will first learn to test for the presence of the three basic organic nutrients. You will then use these tests to identify the organic nutrients in some common foods.

PROCEDURE

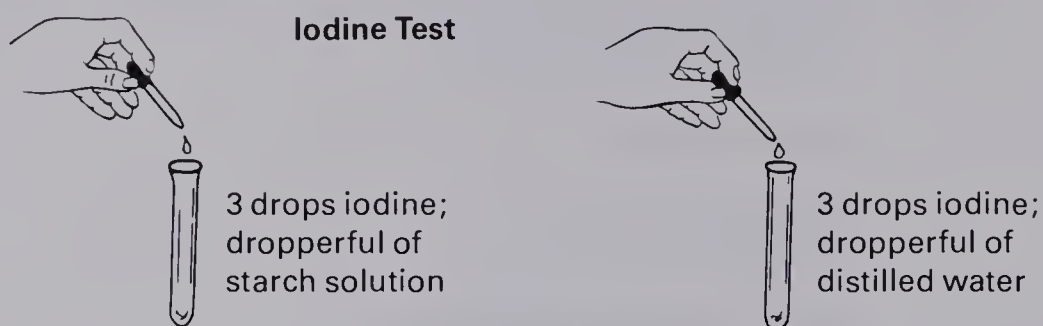
A. Identifying Carbohydrates

Carbohydrates are the organic molecules we call starches and sugars. Simple sugars, such as glucose and fructose, are monosaccharides.

Monosaccharides are the basic subunits, or building blocks from which larger sugars are made. Sugars made up of two simple sugar molecules bonded together are called disaccharides. Starches are known as polysaccharides. They are very large molecules composed of many simple sugar molecules that are bonded together.

Testing for Starch Iodine indicator solution is used to test for the presence of starch. If iodine solution changes its color from brown or orange-red when it is mixed with a substance, it is a positive test for starch in that substance.

Place three drops of iodine into each of two small test tubes. Put one eyedropperful of starch solution into one tube and one dropperful of distilled water into the other. Swirl the tubes to mix the liquids. Observe the color change.

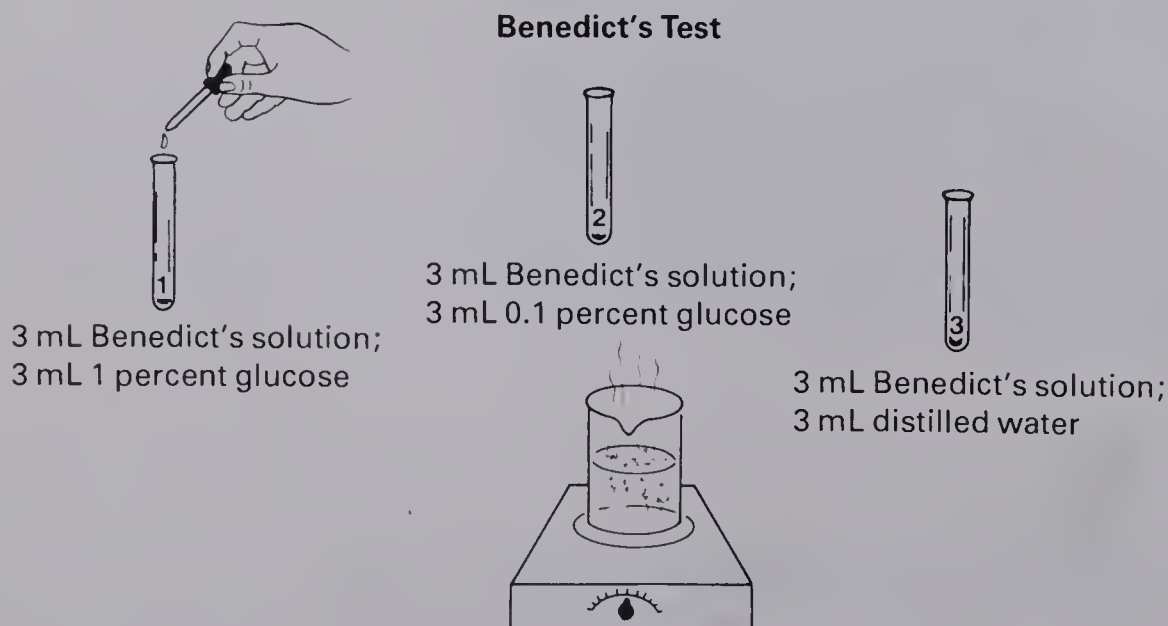


1. What color is the starch and iodine mixture?

2. What color is the water and iodine mixture?

Testing for Sugar Benedict's solution is used to test for the presence of some monosaccharides and disaccharides. Any change in the blue color of Benedict's solution when heated with a substance is a positive test for these sugars.

Obtain three clean test tubes and a 10-mL graduated cylinder. Number the tubes 1, 2, and 3. Pour 3 mL of 1 percent glucose solution into tube 1. Pour 3 mL of 0.1 percent glucose solution into tube 2. Pour 3 mL of distilled water into tube 3. Add 3 mL of Benedict's solution to each of the three test tubes.



Take Care: If you are re-using test tubes, rinse them thoroughly before adding solutions.

Using tongs, place the three tubes in a boiling water bath for three minutes. Note the color changes.

3. What color is tube 1, containing the 1 percent glucose solution?

4. What color is tube 2, containing the 0.1 percent glucose solution?

5. What color is tube 3, the control?

6. Does the concentration of glucose affect the degree of color change in the Benedict's solution?

B. Identifying Lipids

The most common lipids are fats and oils. Fats are solid at room temperature, whereas oils are liquid at room temperature.

Put 1 mL of water and several drops of vegetable oil into a clean test tube. Swirl the tube. Note that the oil and the water do not mix.

7. Does the oil collect on top of or underneath the water?

Obtain a piece of brown grocery-bag paper. With an eyedropper place one drop of vegetable oil and one drop of water on the bag—be certain that the two drops do not touch. Wait a few minutes for the liquids to evaporate. A translucent spot on paper is a positive test for lipids.

8. Did the water stain the bag?

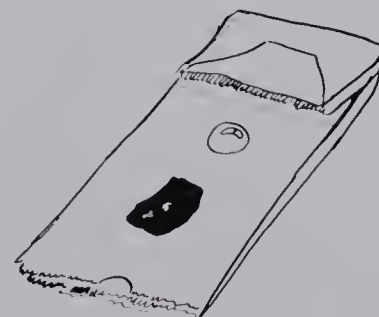
9. Did the oil stain the bag?

C. Identifying Proteins

Concentrated nitric acid is used to test for the presence of proteins. If nitric acid turns a substance yellow, that is a positive test for proteins.

Put about 3 mL of prepared gelatin into a test tube. With an eyedropper, add enough concentrated nitric acid to cover the gelatin. After a few minutes, note the color change.

Caution: Be careful not to get your hands into the hot water of the boiling water bath.



Caution: Nitric acid must be used with great care. It can burn skin and clothing.

10. What color is the protein immersed in concentrated nitric acid?

D. Summary of Tests

11. What is a positive test for starch?

12. What is a positive test for monosaccharides and some disaccharides?

13. What is a positive test for lipids?

14. What is a positive test for protein?

E. Determining Food Composition

Obtain a piece of raw potato and some corn and bean seeds. Chop the food into small pieces to increase the surface area. Devise a method to test each of these foods for starch, sugars, lipids, and protein. If time permits, test other foodstuffs as well.

Fill in the data chart with a plus (+) or a minus (—) to indicate the presence or absence of organic nutrients.

	<i>Starch</i>	<i>Monosaccharide or Disaccharide</i>	<i>Lipid</i>	<i>Protein</i>
potato				
corn				
bean				

ANALYSIS

15. From the data in your chart, what is the main organic component of potato?

16. Which of the materials you tested provides the best source of protein?

17. Which of the three groups of organic compounds we eat provides a ready source of energy?

18. List two uses of lipids in the body.

19. Which group of organic compounds forms enzymes? What do enzymes do?

20. The prefix *mono-* means "one," *di-* means "two," and *poly-* means "many." Why do you think these prefixes are used with the different carbohydrates?

21. Does Benedict's solution test for the presence of all saccharides? If not, which ones does it test for?

22. What is a major difference in the appearance of fats and oils?

23. Did your breakfast, lunch, or dinner yesterday include the three groups of organic compounds? Which foods contained starch, sugar, lipids, and/or proteins?

19 Oxygen and the Growth Rate of Bacteria

PURPOSE

To determine the effects that different amounts of oxygen have on the growth of bacteria.

MATERIALS

broth culture of <i>E. coli</i>	parafilm
2.5 percent boiled lactose solution in airtight container	sterile dropping pipette
2.5 percent boiled lactose solution, aerated	4 sterile 10-mL test tubes
Bromthymol blue	test tube rack
eyedropper	wax pencil

INTRODUCTION

Living things need favorable conditions in order to grow and reproduce. One necessary condition is that the environment provide enough energy to meet the needs of the organisms.

To obtain energy, living organisms break down sugar by cellular respiration. Some organisms need oxygen for this process, some do not need oxygen, and some can carry on the process either with or without oxygen.

The relationship between oxygen and energy can easily be seen by observing bacteria. If the environmental conditions are right, bacteria reproduce in an amazingly short time.

Some types of bacteria are killed by oxygen, and other types can live either with it or without it. The bacterium you will use in this lab, *Escherichia coli* (*E. coli*), is the latter type. This is a common bacterium found in the intestine, and is widely used for research in genetic engineering.

You will grow *E. coli* in environments having different amounts of oxygen. As the bacteria grow, they will use the sugar lactose as a source of energy. The more the bacteria grow and reproduce, the more energy they will require.

PROCEDURE

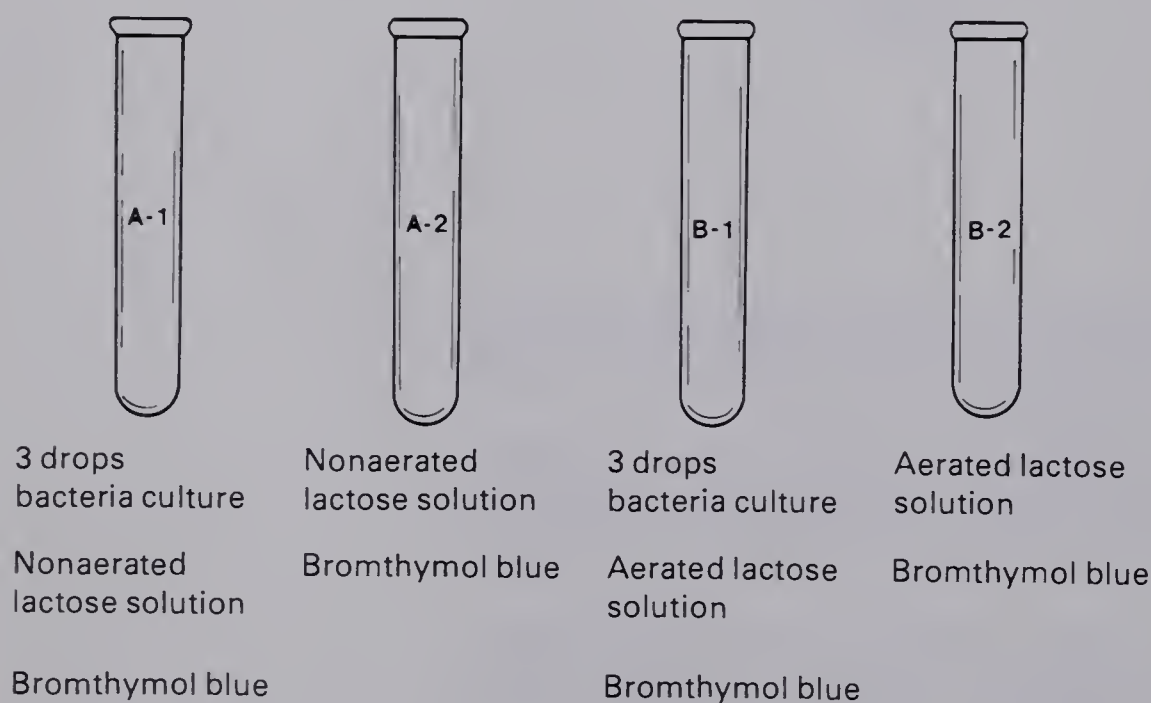
You are going to grow *E. coli* in two lactose solutions. Both solutions were boiled for ten minutes to remove most of the dissolved oxygen. Part of the boiled solution was then transferred to a sterile flask, which was shaken vigorously to reintroduce some oxygen. The remaining solution was sealed to keep out oxygen from the air.

The bacteria will use the lactose in the solution for energy, and give off carbon dioxide as a waste product. As the bacteria increase in number, more carbon dioxide will be produced.

Carbon dioxide forms an acid in lactose solution. The speed at which the solution becomes more acidic can be used as a measure of the rate at which the number of bacteria is increasing. To determine the acidity of the solution, you will use the pH indicator Bromthymol blue. The indicator is a blue color in neutral (pH 7) solutions. It turns to green and then to yellow as the pH lowers and the solution becomes more acidic.

Use a wax pencil to label four sterile 10-mL test tubes "A-1," "A-2," "B-1," and "B-2." With a sterile pipette, place three drops of bacteria culture into tubes A-1 and B-1.

Pour room-temperature, nonaerated lactose solution into tubes A-1 and A-2. Fill the tubes to within 5 mm of the top. With an eyedropper, add to each tube enough Bromthymol blue to produce a distinct blue color (about ten drops).



Seal tubes A-1 and A-2 with parafilm. Gently roll each tube between the palms of your hands until the Bromthymol blue is uniformly distributed. Set the tubes in the test tube rack.

Repeat the procedure for tubes B-1 and B-2, using the aerated lactose solution. Add the same number of drops of Bromthymol blue to these tubes that you added to tubes A-1 and A-2. Seal the tubes.

At the end of the class period, note the color of the tubes and record the data on the chart. Place the tubes in an area specified by your teacher, where they can remain in the dark for 24 hours.

After 24 hours, note the color of the tubes. Record the data on the chart.

Name _____ Date _____

After 24 hours

Boiled lactose
aerated

Meaning of colors: blue blue-green green yellow-green yellow
 _____ more acidic _____

ANALYSIS

1. How do *E. coli* obtain energy?

2. Do *E. coli* grow in the presence of oxygen, in the absence of oxygen, or either way?

3. In the experiment, how did pH indicate the changes in the number of bacteria in the tubes?

4. What relationship did you find between the amount of available oxygen and the growth of the bacteria? In other words, what were your results?

5. State a possible hypothesis for the experiment. Did the results support or disprove the hypothesis?

6. What was the experimental variable in the experiment?

7. Which of the tubes were the controls? What purpose did they serve?

8. Which of the following statements are assumptions for this experiment?

- a. The Bromthymol blue turned yellow.
- b. The bacteria growing in the tubes were *E. coli*.
- c. No other bacteria were growing in the tubes.
- d. Lactose is a sugar.

9. Most animals will die without oxygen. Based upon the information in this lab, what would be the cause of death?

20 Roots

PURPOSE

To study the general structure of roots and to contrast monocot and dicot roots.

MATERIALS

carrot	grass with part of root system
prepared slides of buttercup (<i>Ranunculus</i>) and corn root cross-sections	compound microscope
	knife
germinating radish seedlings	

INTRODUCTION

The root is the underground portion of a plant, and it performs several functions. The root anchors the plant to the ground. It absorbs water and dissolved minerals from the soil and transports them to the upper parts of the plant. It also stores food manufactured by the leaves. Some of the food is used by the root, and some is later transported back through the plant, where it provides material for energy and growth.

PROCEDURE

A. General Structure of the Root

The root has three distinct tissues—the epidermis, the cortex, and the vascular cylinder.

The Epidermis The epidermis is the layer of cells on the outer surface of a root. The epidermis absorbs water and minerals from the soil, with the aid of tiny root hairs. Each root hair is a single cell with a threadlike extension.

Observe the root of a germinating radish seedling. The cottony mass near the tip of the root is made up of root hairs.

1. Do the root hairs extend to the very tip of the root?

As the root grows, new cells form at its tip. Root hairs are produced just behind the growing tip.

2. Draw the root, showing the position of the root hairs.

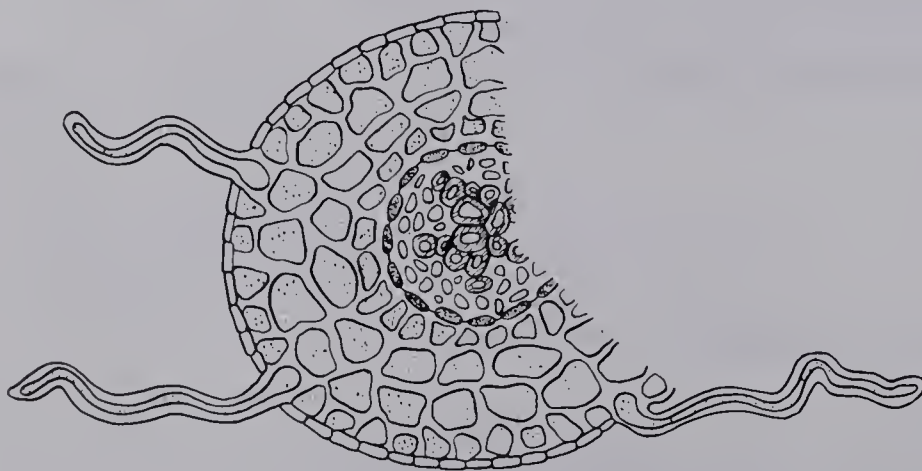
The Cortex Beneath the epidermis is the cortex. The thin-walled cells of the cortex store starch and other food substances.

The innermost layer of cells of the cortex make up the endodermis. These thick-walled waxy cells are tightly packed and form a protective covering over the core of the root.

The Vascular Cylinder The core of the root is the vascular cylinder, also known as the stele. The vascular cylinder contains the two vascular tissues, xylem and phloem. The thick-walled xylem cells conduct water and minerals upward to the stem and leaves. The thin-walled phloem cells conduct food throughout the plant.

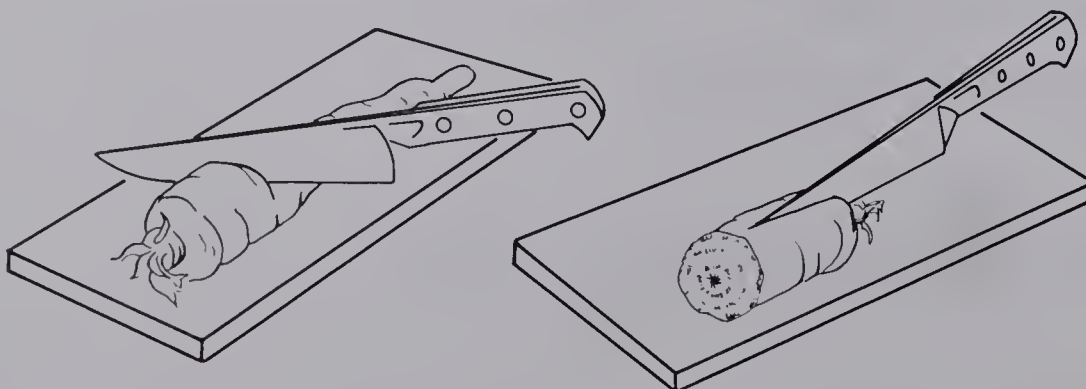
The xylem and phloem are surrounded by one or more layers of cells, known as the pericycle. In many plants, the pericycle cells produce branch roots. Together, the vascular tissues and the pericycle help support the root.

3. On the root cross section shown, label the epidermis, root hairs, cortex, endodermis, pericycle, xylem, and phloem.



Cut a fresh carrot in half with a knife as shown. Then cut the thickest section in half lengthwise. Note the dark orange area—this is the food-storing cortex. The yellow-orange area is the vascular cylinder. (Keep the carrot for use in part B.)

Caution: Cut on a flat surface, in a direction away from your body.



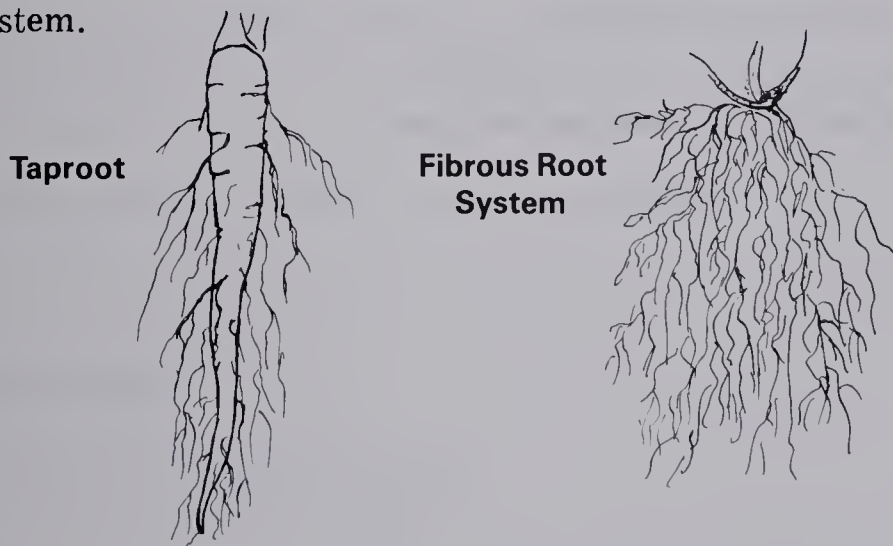
4. Draw the carrot half, showing the cut surface. Label the cortex and the vascular cylinder.

B. Monocot and Dicot Roots

The Root System Dicot and monocot plants can often be distinguished based on the structure of their roots.

In most dicots, the first root that grows out of the seed remains the largest root of the plant. This root is called the taproot. The taproot grows straight downward, and smaller branch roots grow outward from the taproot.

In most monocots, there are many branching roots and no single root is larger than all the others. This root structure is called a fibrous root system.



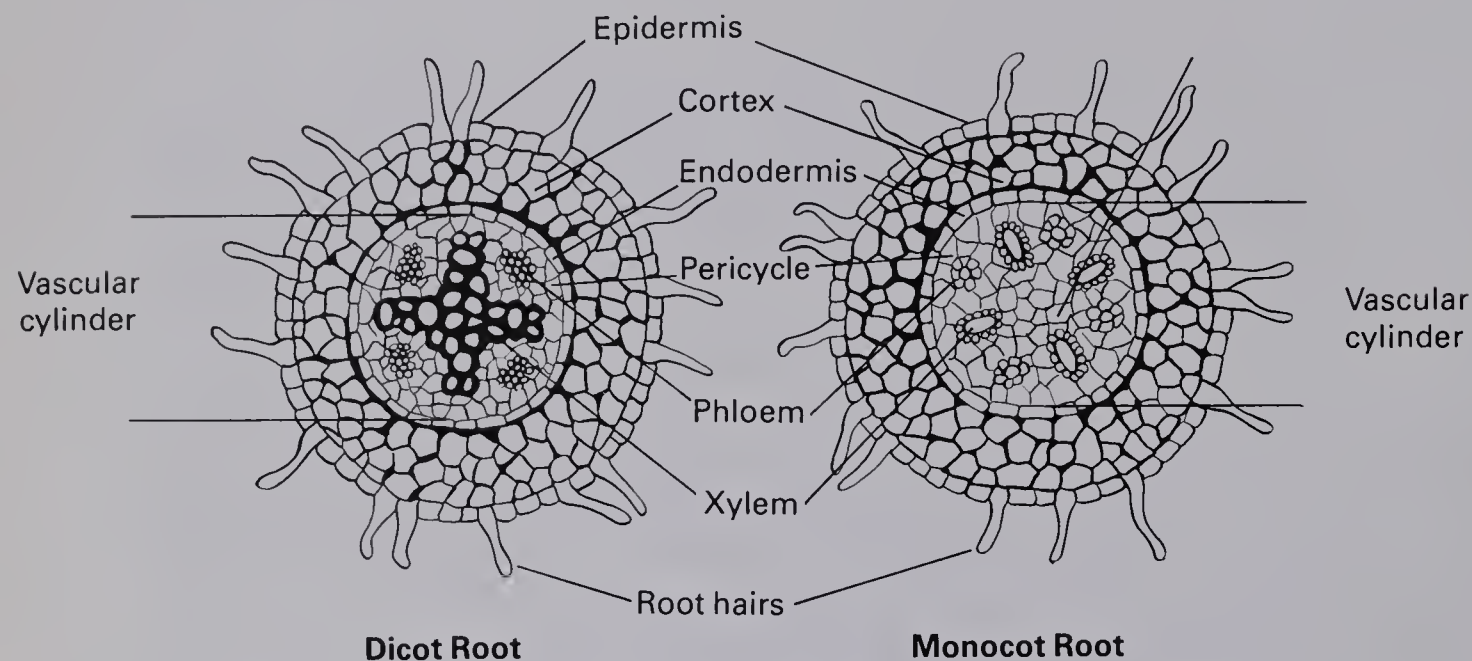
Observe the outside surface of the carrot, and the roots of a patch of grass.

5. Which plant has the taproot? Which has the fibrous root system?

6. Based on differences in their root structures, which is a monocot and which is a dicot?

The Vascular Cylinder Another difference between monocot and dicot roots is apparent in the vascular tissue in the vascular cylinder. In dicots, the xylem cells form a star-shaped pattern. Between the arms of the xylem “star” are the phloem cells. The pericycle surrounds the xylem and phloem.

In monocots, xylem and phloem cells alternate in a ring. The pericycle is located between the endodermis and the xylem and phloem. Inside the ring formed by the xylem and phloem is pith tissue, which aids in storing food and water.



Observe the prepared cross section slides of buttercup and corn roots under the compound microscope. On each, locate the xylem and the phloem. Xylem cells form hollow tubes and in cross section look like empty circles. Phloem cells are smaller and have thinner walls. They contain cytoplasm and so will appear darker than xylem cells.

7. Draw the vascular cylinder of the buttercup root, showing the arrangement of xylem and phloem. Label the xylem, phloem, and pericycle.

Name _____ Date _____

8. Draw the vascular cylinder of the corn root, showing the arrangement of xylem and phloem. Label the xylem, phloem, and pericycle.

9. Which root contains pith? Label it on your drawing.

10. Which root is a monocot and which is a dicot?

ANALYSIS

11. List three functions of a root.

12. What part of the root stores food?

13. What part of the root transports water and minerals? food?

14. List two ways to determine whether a root is a monocot or a dicot.

15. Root hairs absorb water by osmosis. Would a short or a long root hair absorb more water?

21 Stems

PURPOSE

To study the general structure of stems and to contrast monocot and dicot stems.

MATERIALS

prepared slides of corn, alfalfa, and maple (*Acer*) stem cross sections compound microscope

INTRODUCTION

The stem of a plant serves two major functions. It supports the leaves and transports food and water between the roots and leaves. Some stems also play other roles, such as food storage. Green stems contain chloroplasts and manufacture small amounts of food.

Stems are either herbaceous or woody. Plants that live for only a single season are usually herbaceous. Herbaceous stems, though soft and succulent (full of juice), are usually rigid. Water inside the large vacuole of the cells exerts pressure against the cell membrane and cell wall, making the cells rigid.

Woody stems contain strong, woody xylem tissue—the “wood” of a tree. The structure of a woody stem is more complex than that of a herbaceous stem.

PROCEDURE

A. Herbaceous Stems

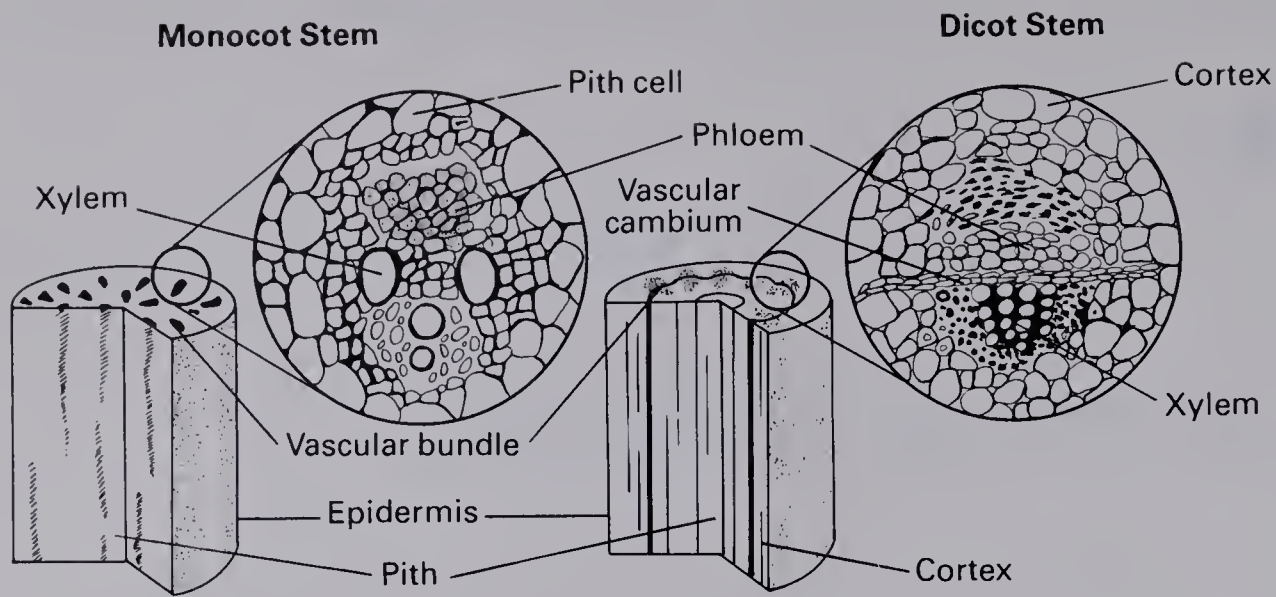
Stems of herbaceous plants show certain differences, depending on whether the plant is a monocot or a dicot.

Cross Section of a Herbaceous Dicot The outer, protective layer of a herbaceous dicot is the epidermis. Beneath the epidermis lies the cortex, which contains chloroplasts and makes some food. The cortex can also function in storing food.

Below the cortex is a layer of vascular bundles. The bundles are tubes of vascular tissue and are arranged in a ring. The outer part of the bundle is phloem tissue, which transports food. The inner part is xylem tissue, which transports water and minerals.

In many herbaceous dicots, the phloem and xylem are separated by a thin layer called vascular cambium. This layer produces new xylem and phloem cells in plants in which secondary growth occurs.

At the center of the stem is the pith tissue, which stores food in some plants.



Examine the prepared slide of an alfalfa stem cross section under the compound microscope.

1. Draw the alfalfa stem. Label the epidermis, cortex, vascular bundles, xylem, phloem, vascular cambium, and pith.

Cross Section of a Herbaceous Monocot The monocot stem differs from the dicot in two ways. The monocot does not have a layer of vascular cambium separating the phloem and xylem. Also, the vascular bundles of the monocot are scattered throughout the stem. Because of this arrangement, the cortex and the pith do not form distinct layers.

Examine the prepared slide of a corn stem cross section under the compound microscope.

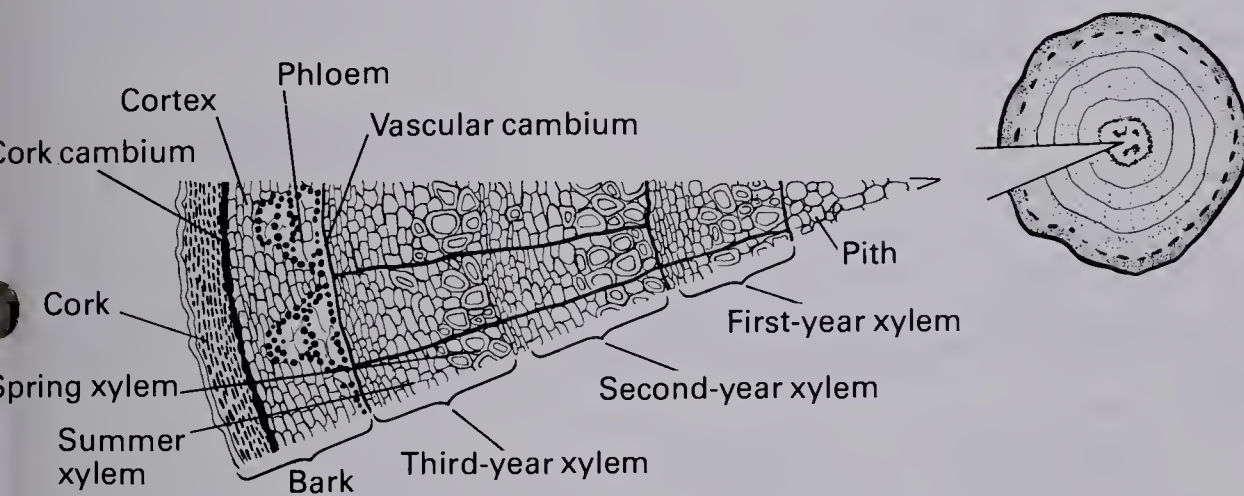
2. Draw the corn stem. Label the epidermis, vascular bundles, xylem, and phloem.

B. Woody Stems

The outer covering of a woody stem is the bark, which has two distinct layers. The outer bark is composed of cork cells, which protect the stem against water loss. Just inside the cork layer is a single layer of cells known as the cork cambium. The cork cambium produces new cork cells. The inner bark contains a layer of cortex, which stores food. The food-conducting phloem tissue is located within the cortex.

Beneath the bark layers is the vascular cambium. This is the growing layer of the stem. If a cambium cell in the outer part of this layer divides, the new cell will be a phloem cell. A new cell formed in the inner part of the vascular cambium layer will be a xylem cell.

Beneath the vascular cambium layer is the water-conducting xylem tissue. This tissue fills much of the stem, forming continuous rings around the central core, or pith, of the stem. The age of many woody plants can be determined by counting the rings of xylem.



On the diagram of the three-year-old stem, notice the arrangement of xylem in each ring. The larger cells are spring xylem. The smaller cells are summer xylem. Spring xylem cells grow larger because more water is available in spring. This size difference creates the appearance of rings, making it easy to tell the spring and summer xylem of one year from that of the next year.

Examine the prepared cross-section slide of the *Acer* stem under the compound microscope. Identify as many of the tissues as you can.

3. How old is the stem? How did you determine the age of the stem?

ANALYSIS

4. What are the two major functions of a stem?

5. How does the arrangement of vascular bundles in a herbaceous monocot differ from that in a dicot?

6. What tissue fills most of a herbaceous stem?

7. What is the function of the vascular cambium in herbaceous dicots? Does it play the same or a different role in woody stems?

8. What tissue fills most of a woody stem?

9. When you remove the bark from a woody stem, the plant will die. Why do you think this is true?

10. In a woody stem with several rings of xylem, where is the newest ring located? the oldest? Why?

11. In a woody stem where are the youngest phloem cells located? the oldest? Why?

22 Cell Size—Is Bigger Better?

PURPOSE

To see the relationship between cell size and diffusion of materials.

MATERIALS

phenolphthalein agar	ruler
0.1 M sodium hydroxide solution	scalpel or knife
250-mL or 400-mL beaker	spoon

INTRODUCTION

The movement of molecules into and out of the cell and throughout the cell is important for the cell's survival. Waste products must be removed. Raw materials must be moved to wherever they are needed. Molecules manufactured by the cell must be relocated.

In order for materials to get into or out of a cell, they must pass through a cell membrane. This membrane is the bridge between the cell cytoplasm and the outside environment. Many molecules cross the bridge by diffusion. Diffusion is the movement of substances from areas of high concentration (where they are abundant) to areas of low concentration (where they are sparse). Movement of many substances within cells also occurs by diffusion.

In this lab you will use cubes of agar as models of cells. Agar is a gelatinous material derived from a marine alga. It is commonly used to cause liquid media to gel.

The agar has been prepared with an acid-base indicator called phenolphthalein. When this indicator comes into contact with a base, such as sodium hydroxide (NaOH), the phenolphthalein turns pink or red.

You will be able to determine how much diffusion takes place by seeing how far the red color penetrates into the cubes.

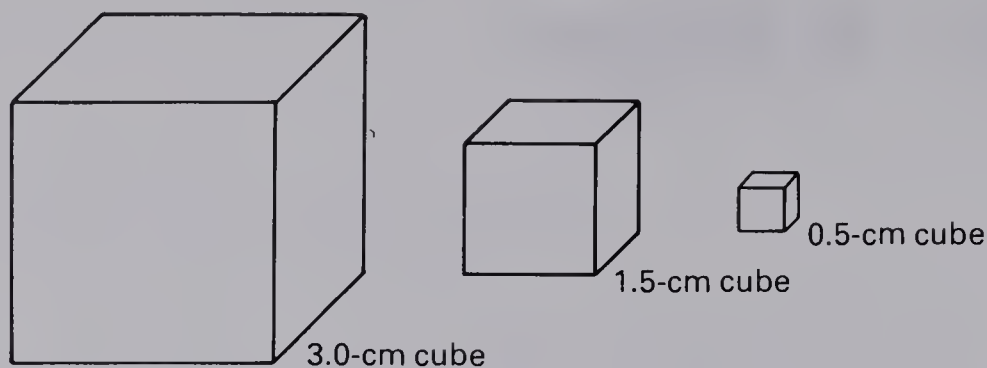
PROCEDURE

A. Diffusion in Cell Models

Obtain a block of phenolphthalein agar. Carefully cut out three agar cubes. Make the first cube 3 cm on a side, the second cube 1.5 cm on a side, and the third cube 0.5 cm on a side. Be sure to cut the cubes in this order, so you will have enough agar for the largest cube. Measure

Caution: Cut in a direction away from your body, and cut down on a hard surface.

and cut the cubes carefully, so that the volume of the cubes can be accurately computed later.



Place the three cubes into an empty beaker, so that the cubes do not touch either the sides of the beaker or one another. Pour in enough sodium hydroxide solution to cover all three cubes. Every two minutes for a period of twelve minutes, carefully turn the cubes with a spoon. Turn the cubes onto a new side each time, to expose all sides to the sodium hydroxide solution.

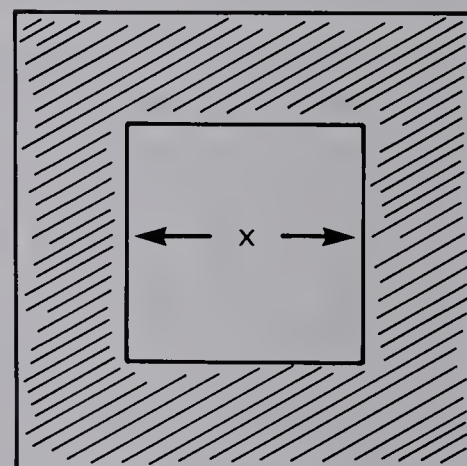
After twelve minutes, use the spoon to remove the cubes from the liquid and place them on a paper towel. Cut each cube in half with a scalpel or knife. Try not to touch the cubes with your hands—steady the cubes with the spoon, if necessary.

You will see how far the sodium hydroxide has diffused into each cube by the red-colored border. Note the uncolored area inside each cube (marked “x” in the illustration). The sodium hydroxide did not diffuse into this area.

In order to determine the percentage of each cube into which the sodium hydroxide did diffuse, you must do some calculations. First, find the volume of each cube. To find volume, multiply length times width times height. In a cube, these three measurements are the same. So, you need to measure only one side and cube it. Volume is given in cubic centimetres (cm^3).

Caution: Sodium hydroxide should be handled with extreme care. Sensitive areas of the body, such as eyes, can be damaged by contact with even weak solutions. Keep your hands clean and dry.

If you get any sodium hydroxide on your hands, immediately rinse them thoroughly in water.



1. Calculate the total volume of the 1.5-cm and 3.0-cm cubes on Data Chart 1. Calculations for the 0.5-cm cube appear on the chart as a guide.
2. Measure one side of the uncolored area of each cube. On the chart, calculate the volume of the uncolored area of the 0.5-cm, 1.5-cm, and 3.0-cm cubes.
3. Determine the volume of the colored area of the cubes by subtracting the volume of the uncolored area from the total volume.

Next, show the volume of the colored area in relation to the total volume of each cube. This can be expressed as a ratio of the volume of the colored area to the total volume.

4. Write the ratios for your cubes on the chart.

Finally, calculate the percentage of each cube into which the sodium hydroxide diffused. Divide the volume of the colored area by the total volume and multiply by 100.

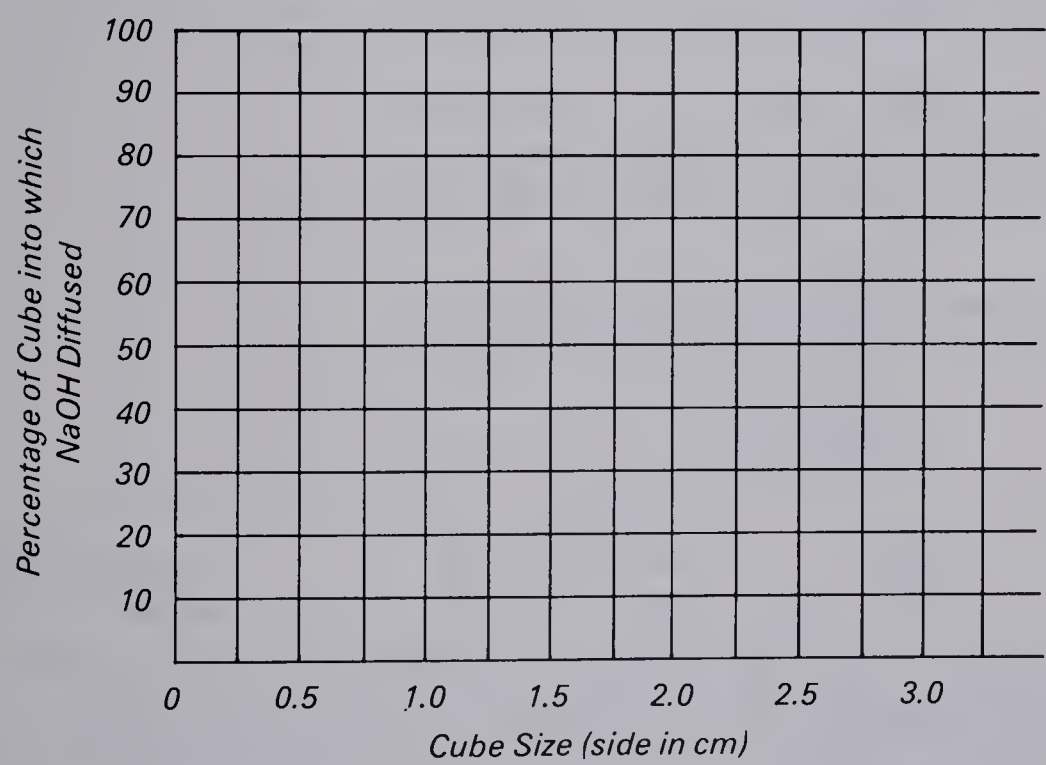
5. On the chart, calculate the percentages for your cubes.

Data Chart 1

Cube	Total Volume of Cube (cm ³)	Volume of Uncolored Area (cm ³)	Volume of Colored Area (cm ³)	Ratio of Colored Volume to Total Volume	Percentage of Cube into which NaOH Diffused
0.5	0.5 × 0.5 × 0.5 = 0.125				
1.5					
3.0					

6. Plot the percentages from the chart on Graph 1. Draw a curve connecting the data points.

Graph 1. Portion of Cube Reached by Diffusion



7. Look at the three cubes. Into which cube did the most sodium hydroxide diffuse? Why?

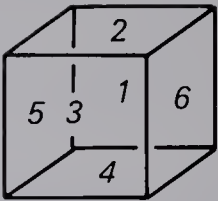
8. Refer to the chart and graph. In which cube was the largest percentage of volume reached by diffusion?

B. Relationship Between Surface Area and Volume

In this section, you will determine the relationship between the amount of surface area a cell has and the volume of the cell. To do this, you will again make some calculations for the three cubes.

To find the surface area of a cube, you must first calculate the area of one of its sides. Multiply length times width—in a cube these measurements are the same.

Next, because a cube has six equal sides, multiply the area of one side by six to find the total surface area of the cube. Total surface area is given in square centimetres (cm²).



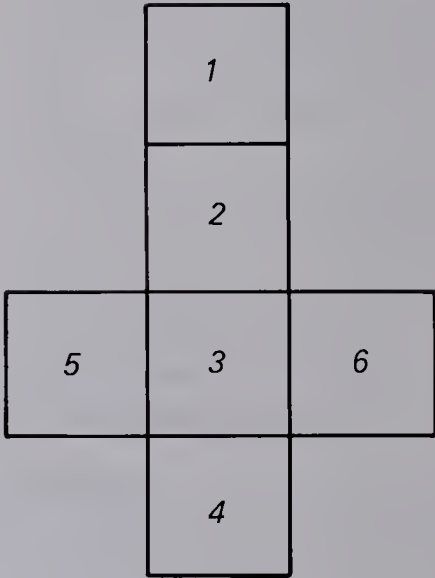
9. On Data Chart 2, calculate the surface area for the 1.5-cm and 3.0-cm cubes. Calculations for the 0.5-cm cube appear on the chart as a guide.
10. On the chart, write the volumes for the cubes (from your calculations on Data Chart 1).

Now that you have found the surface area and volume for the three cubes, show the relationship between surface area and volume as a ratio. For example, the ratio of surface area to volume for the 0.5-cm cube is 1.5/0.125.

The relationship between surface area and volume can also be expressed as a single number called an index. To find the index for the 0.5-cm cube, divide the surface area by the volume: $1.5 \div 0.125 = 12$. The index provides an easy way to compare the surface-area-to-volume ratio in the different cubes.

The index is different for each different size of cube. As the index increases, the amount of surface area per unit of volume increases. Conversely, as the index decreases, the amount of surface area per unit of volume decreases.

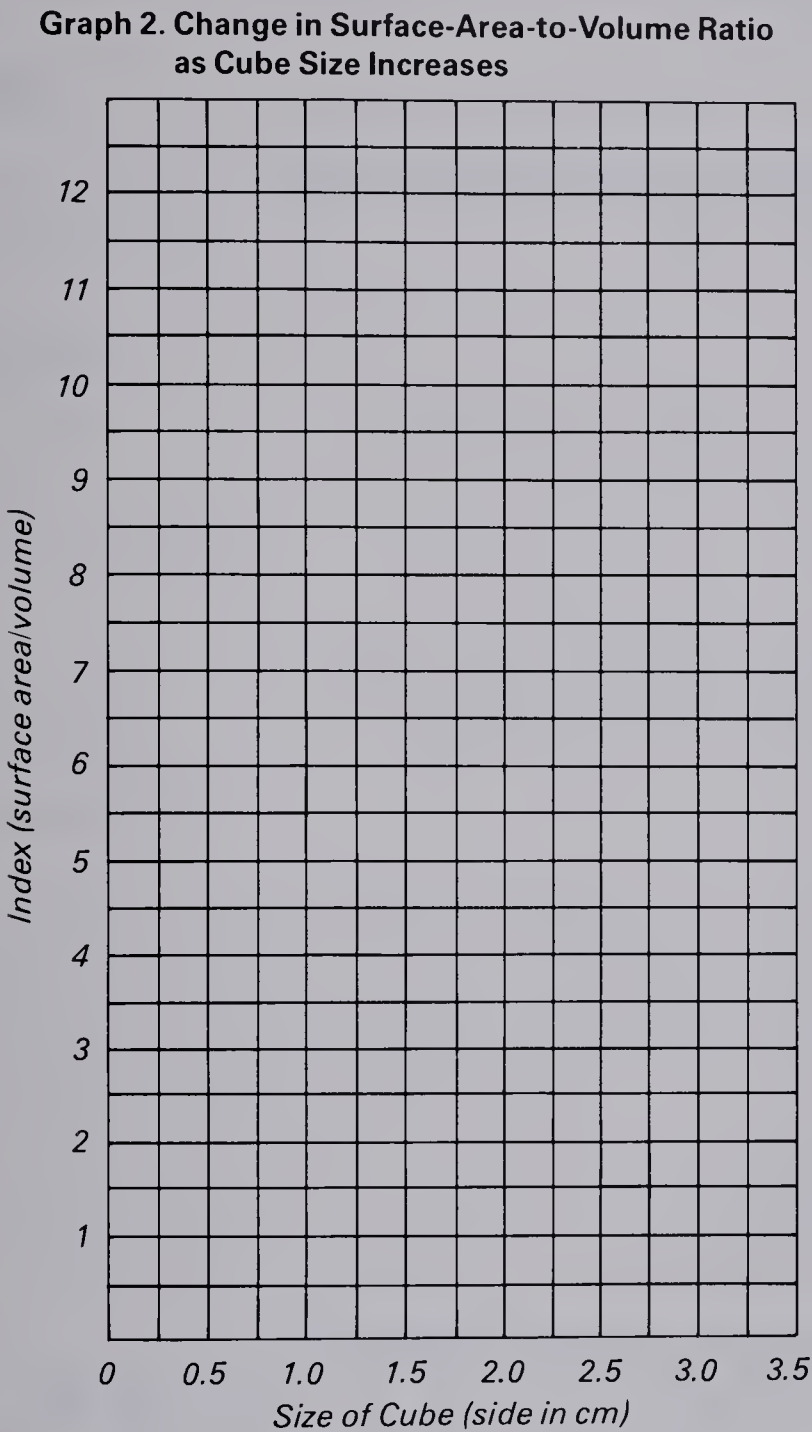
11. On Data Chart 2, write the ratio of surface area to volume and calculate the index for each cube.



Data Chart 2

Cube	Surface Area (cm ²)	Volume (cm ³)	Ratio of Surface Area to Volume	Index
0.5	$0.5 \times 0.5 \times 6 =$ 1.5 cm ²	0.125 cm ³	1.5/0.125	$1.5 \div 0.125 =$ 12
1.5				
3.0				

12. Plot the indexes from Data Chart 2 on Graph 2. Draw a curve connecting the dots.



ANALYSIS

13. Refer to the chart and graph you made in part B. List the cubes in order from largest to smallest. Next, list the cubes from largest to smallest according to their indexes. Are the orders in the two lists the same or the reverse? What does this tell you about the relationship between cube size and index of surface area to volume?

14. Which of the cubes in part B has the greatest surface area in relation to volume?

15. Which of the cubes in part A had the largest percentage of volume reached by diffusion?

16. What relationship do you find between the amount of diffusion and the surface-area-to-volume ratio of a cell?

17. When cells grow to a certain size they frequently divide to form two new smaller cells. Using the information you obtained in this lab, explain why individual cells tend not to grow very large.

18. In part A, did any of the phenolphthalein diffuse out of the cell? How can you tell?

23 How Stomates Function

PURPOSE

To learn how stomates function and how they are affected by the surrounding environment.

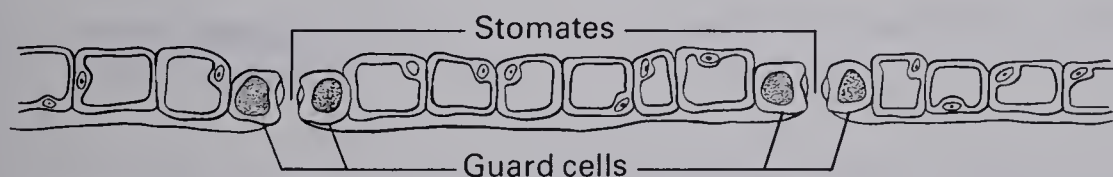
MATERIALS

fresh <i>Zebrina</i> leaf	slides and coverslips
10 percent glucose solution	dissecting needle
cold water	dropping pipette
compound microscope	forceps

INTRODUCTION

The leaf, in order to live and carry out its primary function of photosynthesis, must exchange gases with the atmosphere. Leaf cells involved in photosynthesis use energy from light to convert carbon dioxide and water into glucose and oxygen. In addition, all leaf cells use oxygen and give off carbon dioxide during cellular respiration.

Stomates—pores on the surface of the leaf—play a vital role in gas exchange. Bean-shaped guard cells open and close the stomates, allowing carbon dioxide and oxygen to pass into and out of the leaf. In most plants, the stomates are located mainly on the underside of the leaf.



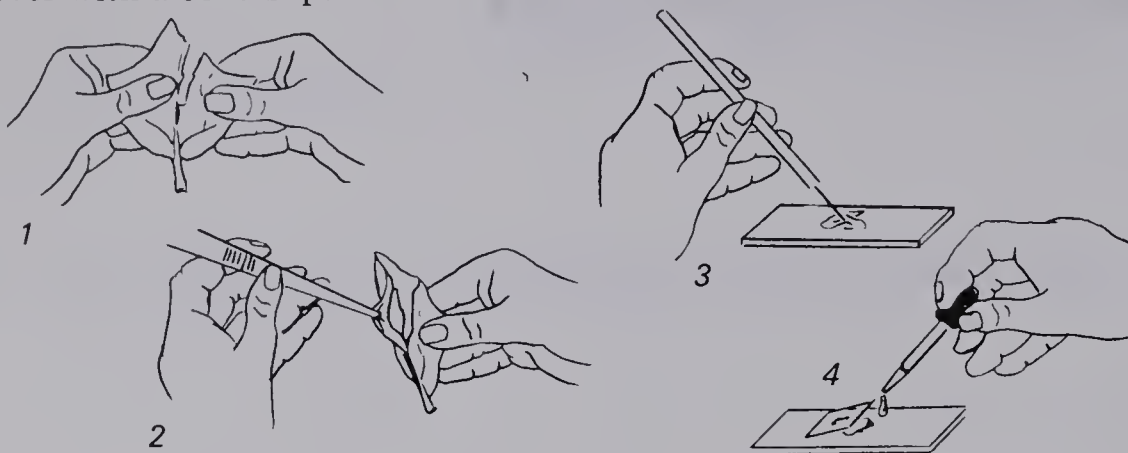
Stomates also regulate the evaporation of water from the leaf. Plants absorb water from the ground through their roots and transport it throughout the stem, branches, and leaves. Water passes out of the stomates into the atmosphere. If the climate is too dry, stomates in some plants close to minimize water loss.

PROCEDURE

You are going to observe the stomates and guard cells on a *Zebrina* leaf. Because they are located on the epidermis, or outer layer, of the leaf, you must make an epidermal peel.

Obtain a leaf that has been soaking in cold water. Bend the leaf until it cracks, but do not tear it all the way through. Make your bend so that the lower epidermis is on the inside. Use a forceps to peel off a

piece of the thin, colorless lower epidermis (on the underside of the leaf). Put the epidermis tissue on a clean slide and straighten any wrinkles with a dissecting needle. Add a drop of water to the tissue and cover with a coverslip.



Stomate Closed



Stomate Open

Observe the epidermis under the compound microscope. The stomates will appear as colorless holes flanked by two green bean-shaped guard cells. The guard cells are green because they contain chloroplasts and carry on photosynthesis.

1. Do any of the other epidermal cells contain chloroplasts?

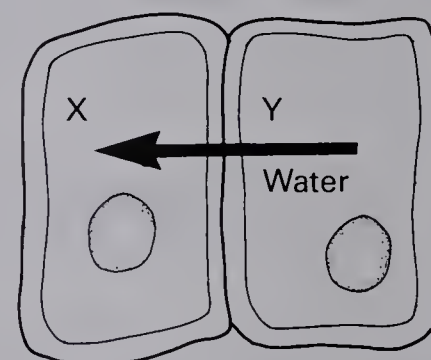
2. Where does photosynthesis occur in the epidermis?

3. What are the two products of photosynthesis?

4. When light shines on the leaf, photosynthesis should be occurring in the guard cells. Do you think the guard cells contain more or less glucose than the other epidermal cells? Why?

If the concentration of glucose is higher in cell X than in neighboring cell Y, water will move from cell Y to cell X. This is because water moves from areas of high water concentration (cell Y) to areas of low water concentration (cell X). The higher the concentration of glucose is, the lower the concentration of water is.

Cell X: high concentration of glucose, low concentration of water



Cell Y: low concentration of glucose, high concentration of water

5. When photosynthesis is occurring in the guard cells, are they full of water or not full of water? Why?

6. When photosynthesis is occurring in the guard cells, are the stomates open or closed? Why?

7. Make a prediction. What would happen to the guard cells and the stomates if you put the epidermis into a solution with a concentration of glucose greater than that in the guard cells?

With a dropping pipette, place one drop of 10 percent glucose solution on the slide so that it touches the coverslip. Touch a paper towel to the other side of the coverslip to draw the solution under the coverslip. Immediately observe the slide under the microscope.

8. What happens to the stomates and guard cells? Was your prediction correct?

At night, photosynthesis stops. The glucose that has been manufactured either moves out of the guard cells and is used by neighboring cells, is transported by the phloem to other parts of the plant, or is converted into starch and stored.

9. When photosynthesis stops, does the glucose concentration in the guard cells increase, decrease, or not change?

10. When photosynthesis stops, does water move out of the guard cells, move into the guard cells from surrounding cells, or not move anywhere?

11. When no photosynthesis is occurring, are the stomates open or closed?

ANALYSIS

12. Are the guard cells full or not full of water when the stomates are open? when the stomates are closed?

13. What changes in the guard cells cause the stomates to open and close? In other words, how do they work?

24 Worm Anatomy

PURPOSE

To become familiar with the anatomy of two kinds of worm.

MATERIALS

- | | |
|-------------------------|------------------|
| live vinegar eels | dissecting pins |
| preserved earthworms | dissecting probe |
| compound microscope | hand lens |
| slides and coverslips | scissors |
| dissecting pan with wax | plastic food bag |
| dissecting needles | |

INTRODUCTION

A worm is a small, soft-bodied animal with no bones. There are many kinds of worms. Some, like tapeworms and flukes, are parasites, which live inside the bodies of other organisms and may cause disease. Other worms, such as earthworms, play an important role in keeping the soil fertile.

In this lab you are going to study two worms—a roundworm, or nematode, and a segmented worm, or annelid. You will examine how these worms perform the functions common to all living things: respiration, digestion, reproduction, circulation, excretion, and locomotion.

PROCEDURE

A. Roundworms

Roundworms have round, smooth, nonsegmented bodies. Although you do not often see roundworms, they occur in extremely large numbers. A shovelful of soil can contain millions of them. Roundworms might even be the most abundant animals on the earth.



The roundworm's body is a "tube-within-a-tube." The tube inside the body acts as the digestive tract.

There is no circulatory system in the roundworm. Instead, blood simply fills the entire body cavity. The roundworm's movement helps to circulate the blood.

The roundworm has one set of muscles, which run longitudinally (lengthwise) under the skin. When muscles are stimulated they contract. After contraction the muscles relax, returning to their original length.

The contracting and lengthening of the nematode's muscles produces a distinctive movement—the trademark of the roundworms. Once you have seen this movement, you will be able to recognize all roundworms by it.

You are going to look at tiny roundworms called vinegar eels. Put a drop of vinegar eel culture on a clean slide and cover with a coverslip. Examine the slide under low power with the compound microscope.

1. Draw one vinegar eel.

2. Describe the locomotion of the vinegar eel.
-

B. Segmented Worms

The earthworm is a segmented worm. Unlike roundworms, segmented worms have distinct rings or segments on their bodies. You will examine both the external and internal anatomy of a preserved earthworm.

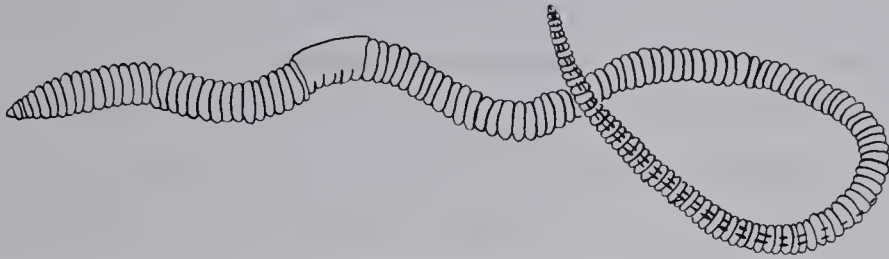
External Anatomy Obtain a preserved earthworm. Place it on a wet paper towel on top of the wax in a dissecting pan.

Each segment of the earthworm has four pairs of short setae, small bristles used for traction in locomotion. If you have ever seen a bird try to pull an earthworm out of the ground, you have witnessed the effectiveness of the setae.

The setae are located on the ventral (under) side of the worm. Run your fingers along the ventral side to feel the stiffness of the setae.

At the anterior (head) end is the mouth. At the posterior (tail) end is the anus, the opening through which undigested food passes out of the body. About one-third of the way from the anterior end is a collar-shaped structure called the clitellum. It produces a mucous cocoon to hold eggs during sexual reproduction.

3. On the drawing of the earthworm, label the mouth, setae, clitellum, and anus.



Internal Anatomy To examine the internal anatomy, you must dissect the worm. The word *dissect* comes from Latin; *dis-* means “apart” and *secare* means “to cut.” Good dissections take time, patience, skill, and sharp instruments.

Place the worm so that its dorsal (back) side is up. Pin the head and tail to the wax. You will first make a *shallow* cut through the skin along the length of the worm. The cut should be off center to avoid cutting the dorsal blood vessel.

Using sharp scissors, make an incision at the posterior end and cut all the way to the anterior end. Be careful to cut through the outer body wall only.

Insert a dissecting needle under the cut skin and carefully separate it from the internal organs. Pin the skin flaps to the wax as illustrated.

As you proceed use the dissecting needles and probe to manipulate the parts of the earthworm.

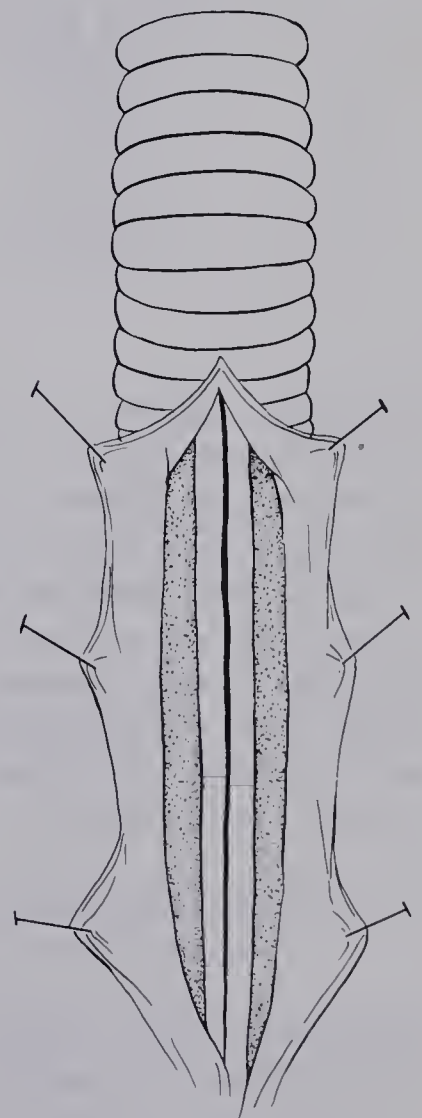
Respiratory System The earthworm has no specialized organs such as lungs or gills to take in oxygen. Instead, oxygen diffuses through its skin into its blood. The skin must be moist for oxygen to move across the cell membranes—if the skin dries, the worm will suffocate.

Muscular System The skin also contains muscles. Just inside the body wall are the circular muscles, which go around each segment. Under these are the longitudinal muscles. When the circular muscles contract, they squeeze the longitudinal muscles, much like a hand squeezing toothpaste out of a tube. The squeezing elongates the longitudinal muscles, which then contract to shorten the worm. The action of these two sets of muscles, together with the setae, makes the earthworm’s locomotion far more efficient than the roundworm’s locomotion.

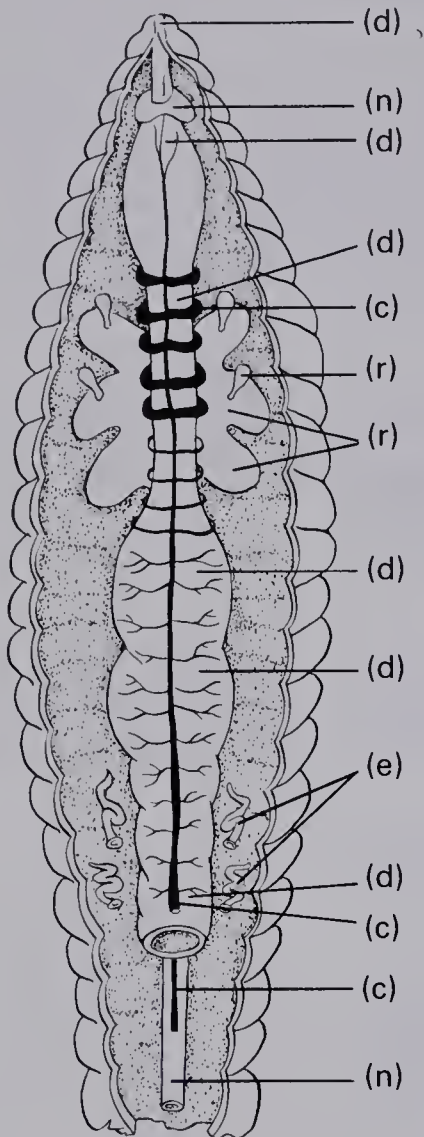
Digestive System Like the roundworm, the earthworm has a tube-within-a-tube body structure. The inner tube is the digestive tract.

Food is taken in by the mouth and swallowed by the pharynx, the widened tube behind the mouth. From there, food travels down the narrow esophagus to the crop. The crop is a large, thin-walled structure that stores food. Just below the crop is the gizzard, a muscular structure that grinds the food. The food is then digested in the narrow intestine, which continues to the posterior end. Any undigested food is removed through the anus.

Caution: Keep your fingers out of the way of the cutting instrument.



4. On the earthworm diagram, label the parts of the digestive system: mouth, pharynx, esophagus, crop, gizzard, intestine, and anus. The systems to which parts belong are labeled on the diagram.



System to which Parts Belong

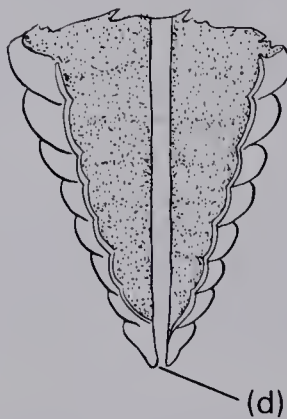
(d) digestive system

(c) circulatory system

(e) excretory system

(r) reproductive system

(n) nervous system



Circulatory System The earthworm has a closed circulatory system, as do humans. Blood is pumped around the body in blood vessels. The blood absorbs digested food from the intestine and oxygen from the skin and delivers them to body tissues.

The blood vessels extend the entire length of the worm. The dorsal vessel is the major pump, moving the blood from the posterior end to the anterior end of the worm. You may find this vessel in the skin flap of the worm. The ventral vessel lies beneath the organs. The two vessels are connected by five aortic arches (sometimes called “hearts”), which surround the esophagus.

5. On the earthworm diagram, label the parts of the circulatory system: dorsal vessel, ventral vessel, and aortic arches.

Excretory System Each segment of the earthworm has its own organs of excretion—the nephridia. The nephridia serve as small kidneys, removing waste products from the blood and body fluids.

The nephridia appear as small white tubes on each side of the digestive tract, near the body walls. You may need to use a hand lens to locate them.

6. On the earthworm diagram, label several nephridia.

Reproductive System The earthworm is hermaphroditic—it has both male and female reproductive organs. The seminal vesicles, which store sperm, are three pairs of white sac-shaped structures on each side of the esophagus. The seminal receptacles, which receive sperm during mating, are two pairs of small white round structures near the vesicles. Testes, which produce sperm, and ovaries, which produce eggs, lie under the seminal vesicles and will probably be difficult to see.

7. On the earthworm diagram, label the seminal vesicles and seminal receptacles.

Nervous System The earthworm has a ganglion mass that serves as its brain. It appears as a small white mass of tissue just above the pharynx. The brain is connected to the ventral nerve cord, which extends the length of the worm.

To see the ventral nerve cord, dissect out a piece of intestine about 4 cm long. The nerve cord appears as a white thread along the ventral body wall. You might see the ventral blood vessel above the nerve cord.

8. On the earthworm diagram, label the brain and ventral nerve cord.

When you have finished the dissection and identified all the parts, ask your teacher or another student to inspect your work.

Remove the pins from the worm. Wrap the worm in the wet paper towel and dispose of it as your teacher instructs.

ANALYSIS

9. Write the system to which each structure listed below belongs. (Systems: respiratory, digestive, circulatory, muscular, nervous, reproductive, excretory.)

anus _____ intestine _____

aortic arches _____ longitudinal muscles _____

blood _____ mouth _____

blood vessels _____ nephridia _____

brain _____ pharynx _____

circular muscles _____ seminal receptacles _____

clitellum _____ seminal vesicles _____

crop _____ skin _____

esophagus _____ ventral nerve cord _____

gizzard _____

10. How do the circulatory and muscular systems differ in a roundworm and an earthworm?

11. How does the earthworm move?

12. What is the function of each of the earthworm's digestive organs?

13. How does the earthworm take in oxygen? With this method of respiration, what kind of environment must the worm live in? Why?

14. What are the four organs that show that the earthworm is hermaphroditic?

25 Starch Digestion

PURPOSE

To test the action of plant and animal enzymes on starch digestion.

MATERIALS

saliva	0.5% starch solution
Benedict's solution	boiling water bath
iodine indicator solution	eyedropper
0.1 percent diastase solution	small beaker
1 percent glucose solution	5 test tubes

INTRODUCTION

In order to convert food into energy, the body must be able to absorb the food molecules. However, just as insects cannot pass through window screens, large food molecules cannot pass through cell membranes. To become small enough to pass through cell membranes, food molecules are chemically broken down by enzymes. This process is called digestion, or hydrolysis.

When starch is digested, or hydrolyzed, it is converted into the simple sugar glucose. Both plants and animals use glucose for cellular respiration. So, both plants and animals have enzymes that hydrolyze starch.

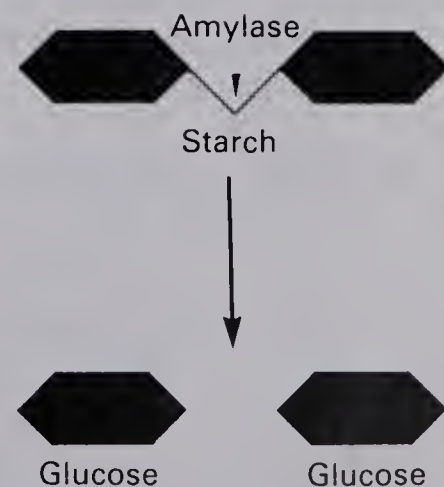
The general name for a starch-digesting enzyme is amylase. The body produces several amylases. The starch-digesting enzyme in saliva is called salivary amylase. Plants also contain several amylases. The mixture of amylases produced by certain germinating seeds is called diastase.

In this lab, you will test substances for the presence of starch and glucose, then test the action of salivary amylase and plant diastase.

PROCEDURE

A. Testing for Starch

The color change that takes place when iodine is added to a substance is a test for the presence of starch. If the iodine produces this color change, starch is present, and the result is said to be positive (+). If the iodine remains orange-red or brown, no starch is present, and the result is negative (—).



Starch Digestion by Amylase

Put 3 mL of starch solution into a clean test tube. Add three drops of iodine indicator solution. Swirl the tube to mix the starch and iodine.

1. Describe the color change that occurs when iodine is added to a substance containing starch.

B. Testing for Glucose

The color change that occurs when Benedict's solution is added to a substance is a test for the presence of glucose. Different amounts of glucose will produce different color changes. The greatest amount of glucose will turn the substance red.

Put 3 mL of glucose solution into a clean test tube. Add 2 mL of Benedict's solution. Mix the solutions by swirling the tube.

Put the tube into a boiling water bath. Watch carefully to observe all the color changes, from the blue color of Benedict's solution in the beginning to the final red color.

2. Describe the color changes that give a positive result in a test for glucose.

C. Testing Saliva and Diastase

Saliva To test for the presence of starch and glucose in saliva, you will follow the procedures described in parts A and B. First, you must collect some saliva. Rinse your mouth with water to remove any sugar that may be there from food you have eaten. Collect about 10 mL of saliva in a small beaker.

With a clean eyedropper, transfer 3 mL (about three droppersful) of saliva to a clean test tube. Add three drops of iodine. Transfer 3 mL of saliva to another test tube and add 2 mL of Benedict's solution. Swirl the tubes to mix; put the tube with the Benedict's solution in a boiling water bath.

3. Does the saliva give a positive or negative result for starch?

4. Does the saliva give a positive or negative result for glucose?

Diastase You will now test for starch and glucose in diastase. Prepare one test tube of 3 mL diastase solution and 3 mL iodine. Prepare another tube of 3 mL diastase solution and 2 mL Benedict's solution. Swirl the tubes to mix; put the tube with the Benedict's solution in a boiling water bath.

Take Care: If you are reusing test tubes from part A, rinse them thoroughly before adding solutions.

5. Does diastase give a positive or negative result for starch?

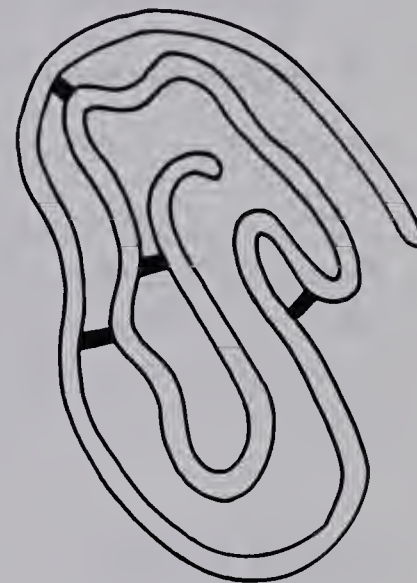
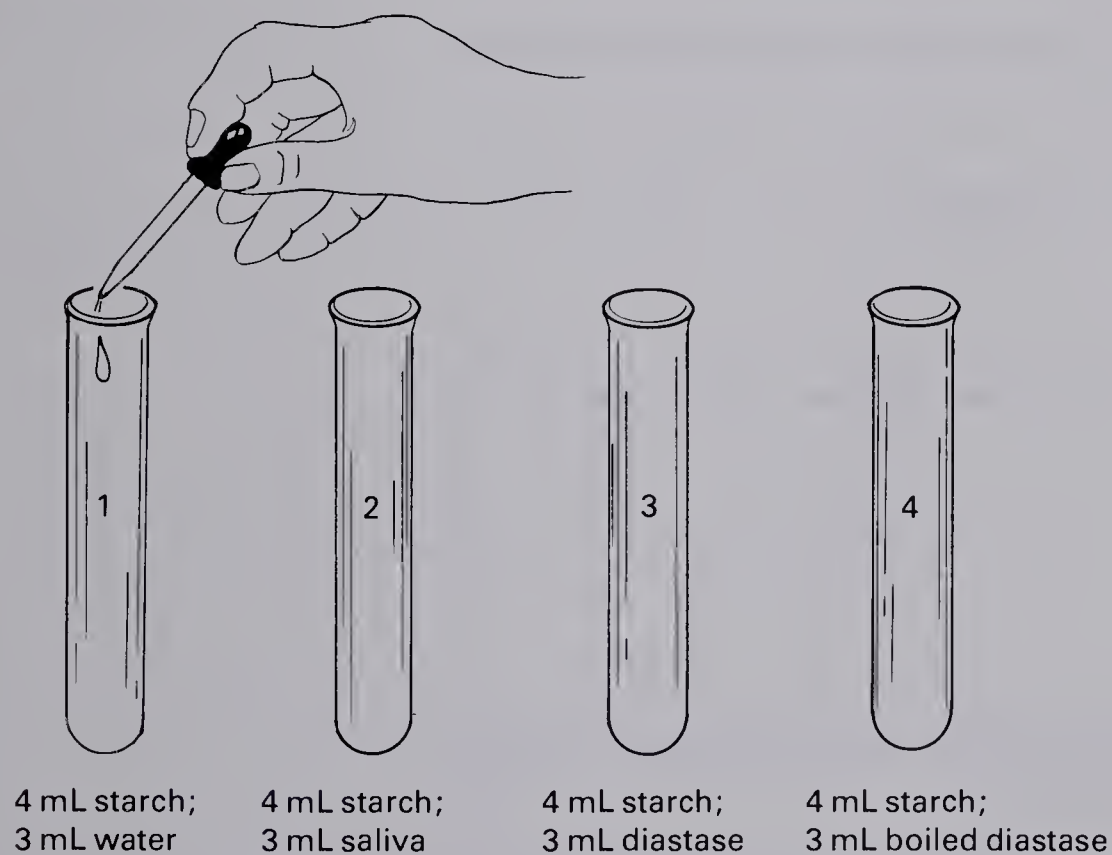
6. Does diastase give a positive or negative result for glucose?

D. Digestion of Starch

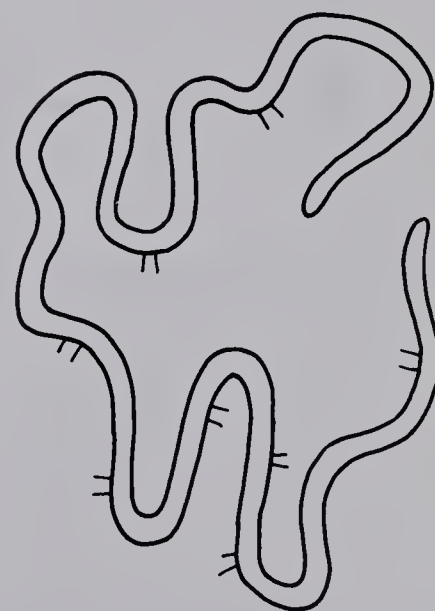
Enzymes are made of large protein molecules. When these molecules are heated, they tend to lose their shape. Protein molecules that have collapsed are called denatured. To determine whether a denatured enzyme can still function, you will test denatured diastase for the presence of starch and glucose. You will also test salivary amylase and diastase for starch and glucose.

To denature diastase, put about 5 mL of diastase solution in a test tube and boil in a boiling water bath. Let the boiled enzyme cool to room temperature before proceeding.

Label four clean test tubes 1, 2, 3, and 4. Prepare the tubes as shown in the illustration. Swirl the tubes well to completely mix the contents. Allow the reactions to occur for about ten minutes.



Enzyme



Denatured enzyme

You will first test each tube for starch. Obtain a clean test tube. Pour about 1 mL of liquid from tube 1 into the clean tube and add a drop of iodine. Mix and note the color change.

Dispose of the liquid in the tube and wash it. Do the starch test for tubes 2, 3, and 4; remember to clean the test tube used for mixing between each test.

Record your results on the data chart. Use a plus (+) or a minus (−) to indicate the presence or absence of starch.

<i>Preparation</i>	<i>Starch</i>	<i>Glucose</i>
1 Starch and water		
2 Starch and saliva		
3 Starch and diastase		
4 Starch and boiled diastase		

Now you will test tubes 1, 2, 3, and 4 for glucose. Pour one drop of Benedict's solution into the liquid remaining in each tube. Put the tubes in a boiling water bath and observe any color changes.

Record your results on the data chart. Indicate relative amounts of glucose using one, two, three, or four pluses.

ANALYSIS

7. In which tube (in part D) was the starch digested most effectively?

8. Which tube or tubes had no glucose?

9. Why did some tube or tubes have glucose, while others did not?

10. Did boiling have any effect on the enzyme's ability to hydrolyze starch? If so, what was the effect?

11. In part C, did your saliva alone contain glucose? If so, where might it have come from?

Name _____ Date _____

12. In part C, did the diastase solution alone contain glucose? If so, where might it have come from?

13. How would the presence of glucose in either the saliva or diastase affect the outcome of the experiment in part D?

14. Tube 1 in the experiment was the control. Why was this tube necessary?

15. Why is it necessary for the body to hydrolyze starch?

26 Protein Digestion

PURPOSE

To study conditions that are favorable for protein digestion.

MATERIALS

fresh egg white	12-cm-long, 2-mm-bore, fire-polished capillary tubing
0.1 M hydrochloric acid	forceps
0.5 percent pepsin solution	metric ruler
4 beakers, 50 mL or larger	triangular file
pan of boiling water	

INTRODUCTION

To absorb proteins, the body must break them down into pieces small enough to pass through cell membranes. This breaking-down process is called digestion, or hydrolysis.

Proteins are chains of amino acids linked by chemical bonds. During protein digestion, enzymes break the bonds between amino acids, producing small polypeptide chains and individual amino acids. When digestion is complete, all the small polypeptide chains and proteins have been broken down into amino acids.

In humans, digestion begins in the stomach. Hydrochloric acid and the enzyme pepsin are both present in the stomach. In this lab, you will investigate whether hydrochloric acid alone, pepsin alone, or both are needed to digest protein.

PROCEDURE

You will observe the rate of digestion of the proteins in egg white. Prepare the egg white in the following way.

Using a piece of fire-polished capillary tubing like a straw, draw raw egg white into the entire length of the tubing. When the tubing is filled, immediately place your finger over one end to hold in the egg white. Carefully put the tubing—uncovered end first—into a pan of rapidly boiling water. Do not let your hand touch the water. The egg white should quickly coagulate, and so will not run out of the tubing.

When all the egg white has become solid, remove the tubing from the water with forceps. Let the tubing cool until it can be handled.



Caution: Be careful when handling the capillary tubing. Rough ends are sharp enough to cut skin.

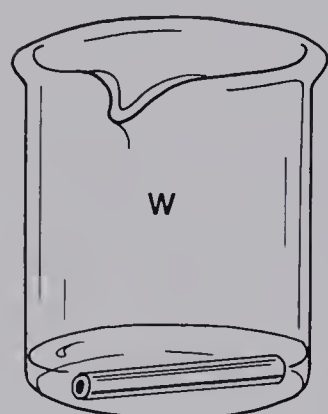
Cut the tubing into four pieces each about 3 cm long (do not remove the egg white). To cut, use a file to make a deep scratch across the tubing. Wrap the tubing in a paper towel. Place your thumbs on opposite sides of the scratch and gently snap the tubing.

The egg white should be flush with the end of the tubing. Trim off any excess.

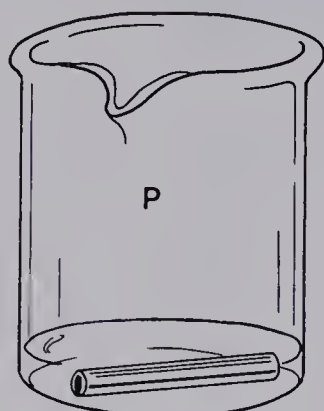
Place one piece of tubing on its side in each of four beakers. Fill each with enough of the following fluids to cover the tubing.

Pour water into the first beaker and label it "W." Pour pepsin into the second and label it "P." Pour hydrochloric acid into the third and label it "A." Pour equal amounts of pepsin and hydrochloric acid into the fourth beaker and label it "PA." During the rest of the class period, periodically swirl the beakers so the fluid will come in contact with the egg white.

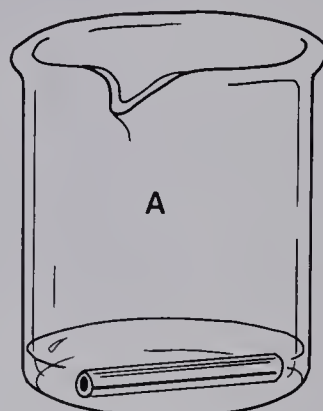
Caution: Hydrochloric acid can damage skin and clothing. If you get any acid on you, rinse thoroughly with water.



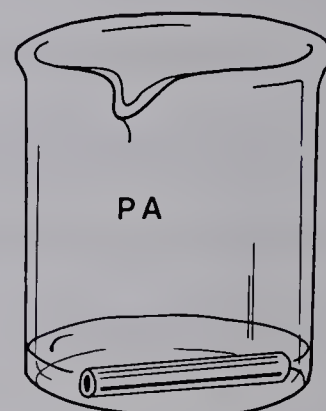
Water



Pepsin



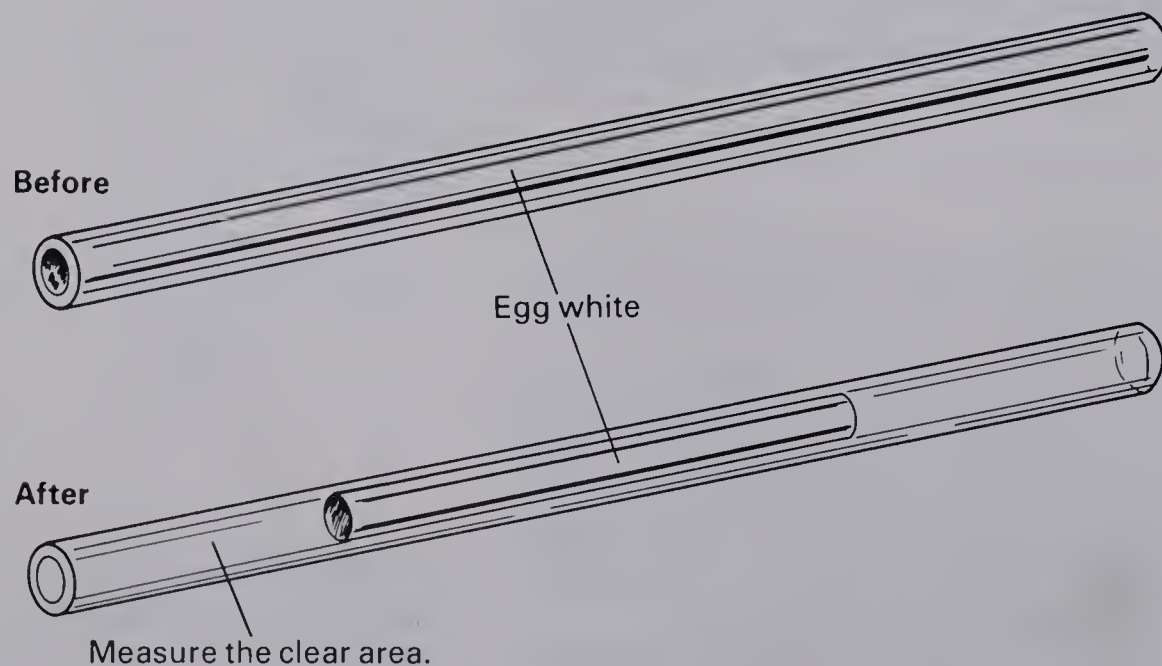
Hydrochloric acid



Pepsin and hydrochloric acid

Every ten minutes until the end of the period, take a reading of the tubes. Use forceps to remove the tubes from the beakers. Place the tubes on a paper towel, taking care not to touch them with your hands.

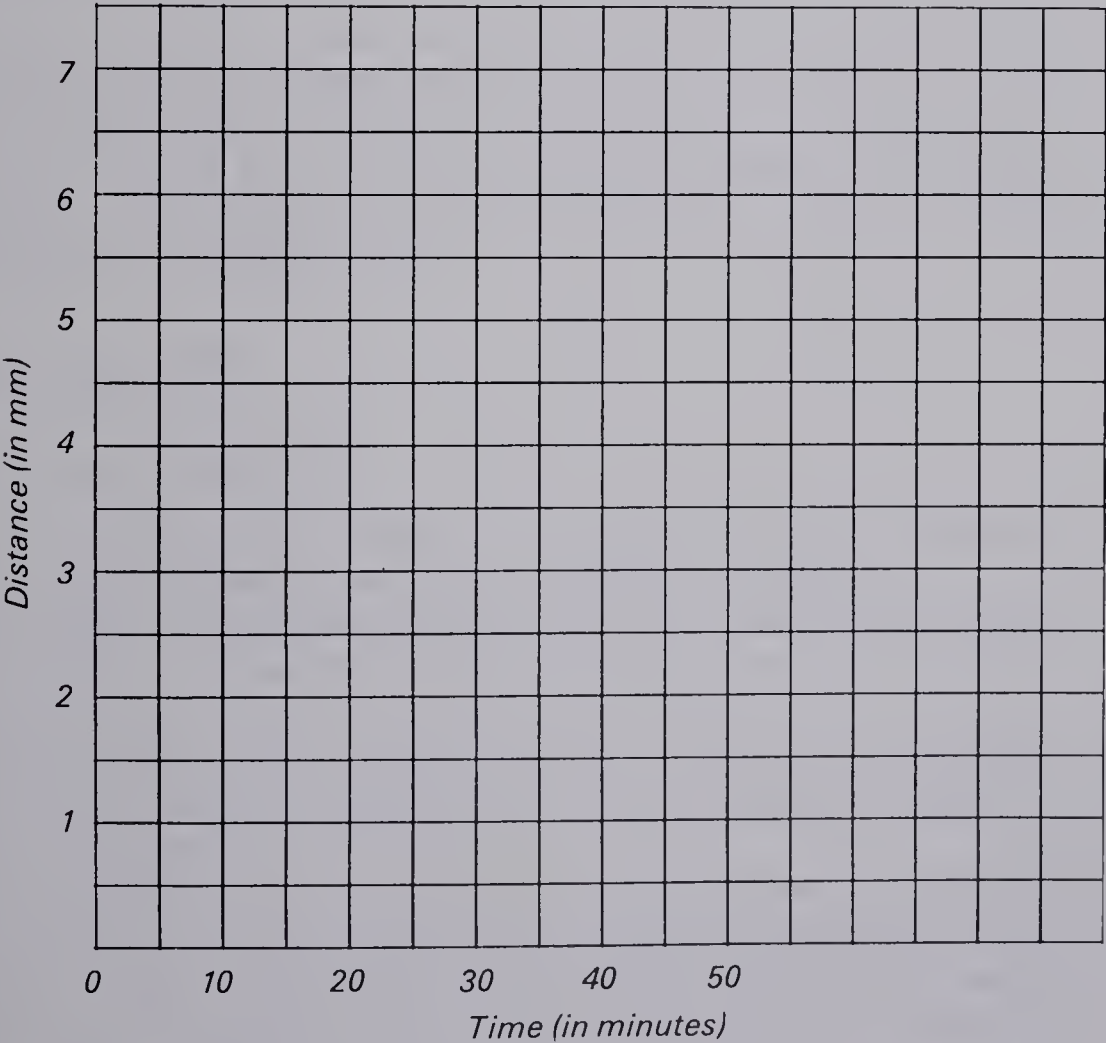
With a ruler, measure the distance in millimetres from one end of the tube to the end of the solid egg white. This distance shows how much of the protein has been digested. When egg white is digested, it changes from a white solid to a clear liquid. Use forceps to replace the tubes in their beakers. Measure the same end of the tubes each time you take a reading.



1. After each reading, record the measurements for the four tubes on the chart.

Tube	Distance Between Egg White and End of Tube (in mm)			
	10 min.	20 min.	30 min.	40 min.
W				
P				
A				
PA				

2. Plot the data from the chart on the following graph. Use a different color pencil to mark the readings for the different tubes. Label each line with the letter of the tube it represents.



ANALYSIS

3. In which solutions did digestion occur?

4. Which solution was most effective in digesting the protein?

5. What was the purpose of the beaker with water?

6. Based upon this experiment, what conclusion can you make about the conditions necessary for protein digestion?

27 Feeding Methods

PURPOSE

To observe food-gathering and ingestion in three different types of organisms.

MATERIALS

cultures of <i>Paramecium</i> , <i>Hydra</i> , <i>Planaria</i> , and <i>Daphnia</i>	dissecting microscope
	slide and coverslip
boiled yeast stained with congo red	eyedropper
methyl cellulose solution	forceps
raw liver	toothpick
compound microscope	watch glass

INTRODUCTION

Organisms that cannot make their own food are called heterotrophs. They obtain food by eating other living things. In this lab you will observe three kinds of animals in the processes of food-gathering, ingestion (taking in food material), and digestion. There are two kinds of digestion. Intracellular digestion occurs inside of a cell. Extracellular digestion occurs outside of a cell.

PROCEDURE

A. Feeding in *Paramecium*

The first organism you will observe is a paramecium. The paramecium is unicellular and free-swimming (not attached to anything).

Put a drop of the *Paramecium* culture on a clean slide and add a drop of yeast that has been stained with congo red. Do not add a coverslip.

Observe the slide under the lowest power of your compound microscope. Notice how the paramecia swim around the slide. Are they ingesting the yeast?

After a couple of minutes, remove the slide from the stage. Now that the paramecia have started ingesting the yeast, slow them with methyl cellulose in order to observe them more easily. Add a drop of methyl cellulose to the slide and with a toothpick gently mix it into the liquid containing the paramecia.

Take Care: Do not let the microscope lens touch the liquid on the slide.

Return the slide to the stage of the compound microscope and observe it again under low power. If you wish to use high power, you must first add a coverslip.

Observe one paramecium that is feeding. Note how the red yeast cells are ingested by way of a depression on one side. This is the oral groove. The food is then pushed along to the mouth, where chambers called food vacuoles form around the food. Note how these vacuoles travel around the inside of a paramecium.

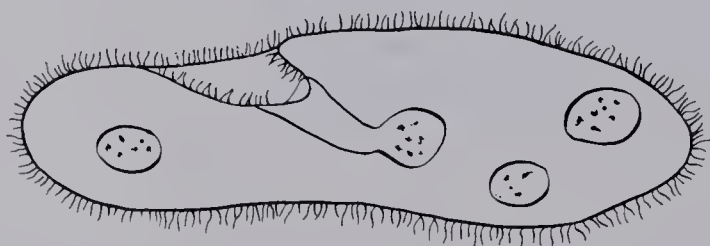
Digestion takes place in the food vacuoles. Since the food enters the cell before being digested, digestion is intracellular.

Watch the digestive process in the food vacuoles for several minutes. To digest the yeast, the paramecium produces digestive enzymes. As the enzymes break down the food, the solution in the vacuoles becomes acidic. Congo red changes color in the presence of an acid. Observe the yeast until you can see a color change.

1. Are food vacuoles formed in one place or in many places on the surface of the paramecium?

2. What color does the stained yeast become as it is being digested?

3. With an arrow, show on the outline of the paramecium where food is ingested. Label the oral groove. Draw and label as many food vacuoles as you saw on the live paramecium.



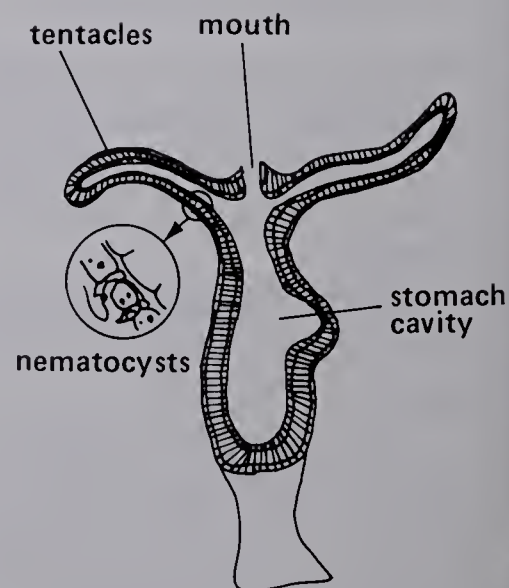
B. Feeding in *Hydra*

The hydra is a stationary multicellular animal. It attaches itself to water plants, stones, and sticks—or in this case, to the culture dish.

With an eyedropper, carefully remove a hydra from the culture and place it into a watch glass. You must act quickly or the hydra will attach itself to the wall of the eyedropper. Add enough water to the watch glass to cover the hydra.

Observe the hydra under the dissecting microscope. Notice how it moves the tentacles around its mouth. The tentacles contain special stinging cells called nematocysts.

Add a few small daphnia to the watch glass. Observe how the hydra catches and ingests the daphnia.



4. How does the hydra catch its prey?

5. Describe the process of ingestion. Is more than one tentacle involved?

The stomach, or digestive cavity, of the hydra is shaped like a cup. If you have time, observe a daphnian as it is ingested into the digestive cavity and then digested. Extracellular digestion of large food particles occurs in the digestive cavity. The partially digested particles then pass by endocytosis into the cells lining the digestive cavity. Intracellular digestion occurs here.

6. Draw a hydra ingesting a daphnian.

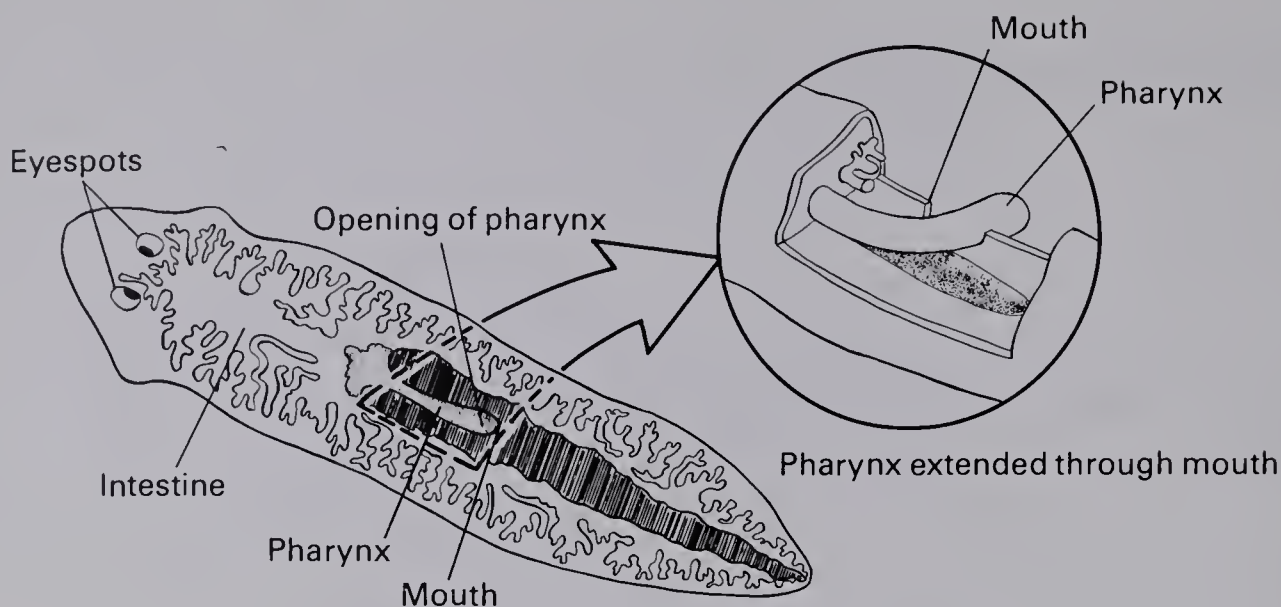
1	2
3	4

C. Feeding in *Planaria*

The planarian is a flatworm. It is multicellular and able to move about freely in water.

Using forceps, place a planarian in a watch glass. Add enough water to fully cover the planarian, allowing it to move about. Observe the planarian under the dissecting microscope. Illuminate with room light or other soft light source. Avoid using bright light, which will disturb the planarian.

Look at the planarian's dorsal (top) surface. The two spots that look like crossed eyes are light-sensitive **eyespot**s.



With a toothpick, turn the planarian over and look at its ventral (underside) surface. The tube that protrudes is called the **pharynx**.

The pharynx is connected to a tube called the intestine, which branches throughout the animal. Most of the food is digested by extracellular digestion in the intestine. Intracellular digestion also occurs in the cells of the intestinal lining.

Put a small piece of raw liver into the watch glass, *away* from the planarian. Observe the behavior of the planarian as it finds the food and ingests it.

7. What structure does the planarian use to ingest the liver?

8. Draw the planarian ingesting the liver.

ANALYSIS

9. What kind of digestion, intracellular or extracellular, takes place in the paramecium, hydra, and planarian?

10. What structures on the paramecium, hydra, and planarian are used for food-getting and ingestion? What structures are used for digestion?

11. What is the major difference between the way a paramecium gets food and the way a hydra and a planarian get food?

12. What is the major difference between the way a hydra and a planarian get food?

13. Is there a limit to the size of food that can be eaten by paramecia, hydras, and planaria? Why or why not?

14. Why would it be unlikely for an organism like the paramecium to have extracellular digestion?

28 Osmosis

PURPOSE

To demonstrate the process of osmosis.

MATERIALS

raw chicken egg with shell removed

distilled water

balance

5 percent salt solution

3 beakers

10 percent salt solution

spoon

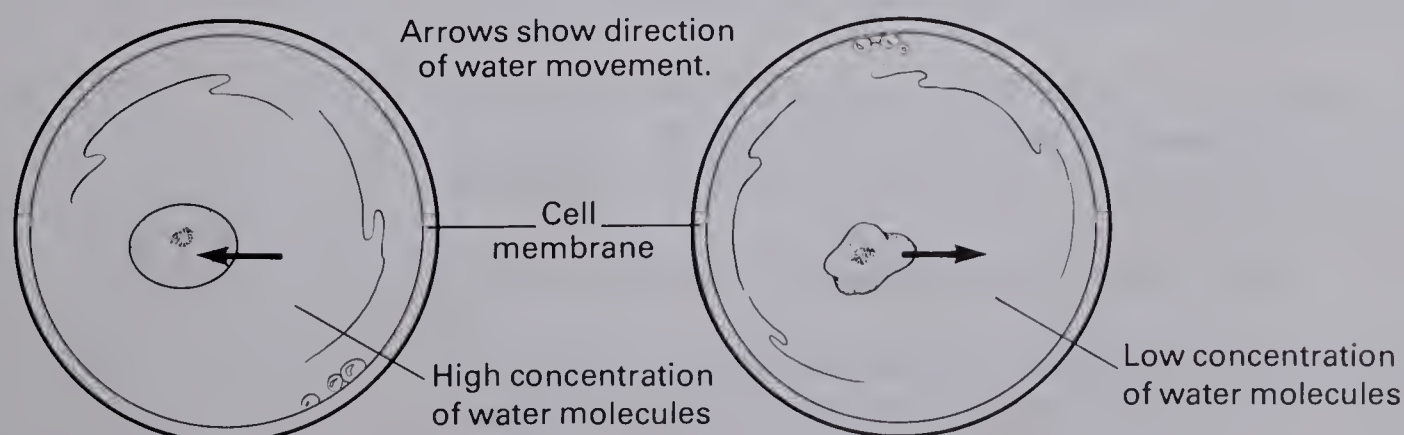
distilled water

INTRODUCTION

Water is the most abundant substance in any cell. All the chemical processes of the cell involve water in some way. Water passes into and out of the cell by osmosis. Osmosis is the diffusion of water through a semipermeable membrane from an area where the concentration of water is high to an area where the concentration of water is low.

The cell membrane is a semipermeable membrane. It allows some substances to pass through while blocking others. Because water molecules are relatively small, they easily pass through the cell membrane. If the cell is in an environment where the concentration of water molecules is greater outside the cell than it is inside, water will move through the membrane into the cell by osmosis. If the concentration of water is greater inside the cell than it is outside, the water will move out of the cell by osmosis.

The amount of water in the cell changes as the cell's environment changes. If too much water enters the cell by osmosis, the cell may burst. If too much water leaves the cell, the cell will shrink. In the normal environment of a cell, however, the water concentration does not undergo such radical changes.



In this lab you will use a raw chicken egg with the shell removed as a model of the cell to demonstrate the process of osmosis. The egg membrane is semipermeable, as is the membrane of a cell.

PROCEDURE

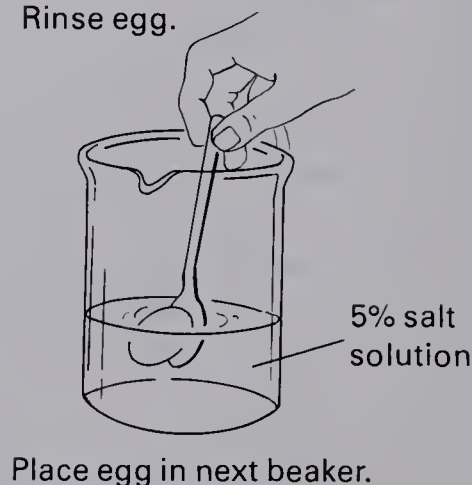
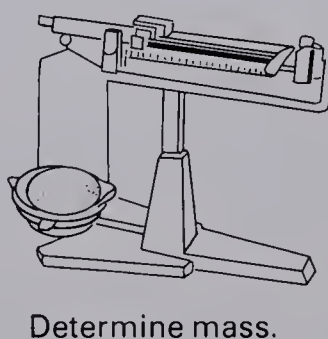
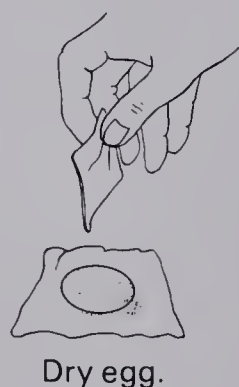
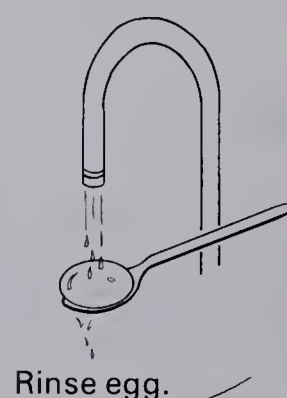
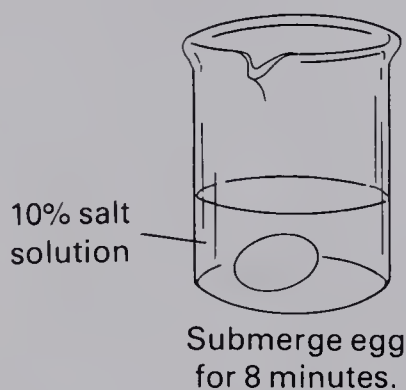
The eggshell was removed by placing the egg in dilute hydrochloric acid overnight. The acid dissolved the shell, but left intact the membrane surrounding the yolk and white. If any shell remains on your egg, do not remove it. It will not interfere with the experiment.

Pick up an egg with the spoon and carefully rinse it under tap water. Place it on a paper towel on a table, and gently pat it dry with another paper towel.

Determine the mass of the egg on a balance and record the mass on the data chart.

Place the egg in a beaker containing 10 percent salt solution. Make sure there is enough liquid to cover the egg. Leave it in the solution for eight minutes. Then remove the egg with the spoon and pat it dry as you did before. Determine the mass of the egg and record it on the data chart.

Take Care: The egg is raw, so handle it carefully. Although the egg membrane is tough, it can break if the egg is handled roughly.



Rinse the egg gently in tap water and place it in a beaker containing 5 percent salt solution for eight minutes. Then remove the egg with the spoon and pat it dry. Determine the mass of the egg and record it on the chart.

Rinse the egg gently in tap water and place it in a beaker containing distilled water for eight minutes. Remove the egg with the spoon and pat it dry. Determine the mass of the egg and record it on the data chart.

When you are finished, return the egg to the place specified by your teacher.

Calculate the changes in the mass of the egg on the data chart. Pool your data with the rest of the class and calculate the class averages.

Individual Data

	<i>10% salt solution</i>	<i>5% salt solution</i>	<i>Distilled water</i>
Starting mass			
Final mass			
Change in mass (+ or -)			

Class Data (average)

Starting mass			
Final mass			
Change in mass (+ or -)			

NOTE: The starting mass of the egg in the 5 percent solution is the same as the final mass in the 10 percent solution. Also, the starting mass of the egg in distilled water is the same as the final mass in the 5 percent solution.

- How do your data compare with the class data?

ANALYSIS

- Define osmosis.

- [illegible]

-

-

-
-
-
-
-

29 Movement of Materials Across Cell Membranes

PURPOSE

To investigate the movement of materials into and out of cells and to demonstrate plasmolysis.

MATERIALS

- | | |
|------------------------------------|-----------------------|
| <i>Elodea</i> (<i>Anacharis</i>) | compound microscope |
| yeast culture (fresh and boiled) | slides and coverslips |
| congo red solution | eyedropper |
| 20 percent salt solution | |

INTRODUCTION

As the liquid environment around a living cell changes, several things may happen. One possibility is that dissolved materials will move into or out of the cell by diffusion. The movement of dissolved materials is usually accompanied by the movement of water by osmosis. In both diffusion and osmosis, the cell does not expend any energy. Therefore, these processes are called **passive transport**.

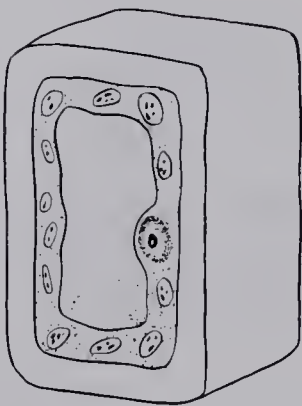
Another possibility is that the cell will selectively move certain materials in or move certain small molecules and ions out after they diffuse in. Because the cell expends energy in this process, it is called **active transport**.

Still another possibility is that dissolved materials will simply stay outside or inside the cell because they are too large to pass through the cell membrane or the cell wall.

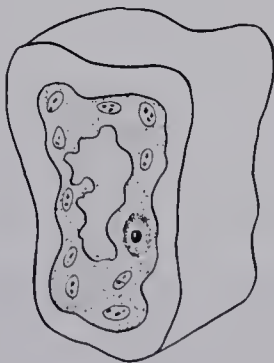
The concentration of dissolved materials in and around the cell determines how much water is inside the cell. This is true because water always moves passively by osmosis in the direction of low water concentration. If materials move into the cell, the concentration of water in the cell lowers. If materials move out, the water concentration rises.

The amount of water in a cell greatly determines the cell's shape. When a cell is full of water, it becomes rigid and is said to be **turgid**. This is the normal cell state. When a cell is not full of water, it becomes limp and is said to be **flaccid**.

When a cell becomes so flaccid that the cytoplasm shrinks, the cell is said to be **plasmolyzed**. The withdrawal of water from a cell, or



Turgid



Flaccid

plasmolysis, causes the cytoplasm to shrink, giving a wrinkled appearance to the cell membrane.

In this lab you will perform a test to determine whether active transport has taken place. You will also test the effect of salt solution on plant cells.

PROCEDURE

A. Movement by Active Transport

Obtain two clean slides. Mark an "A" on one and put a drop of fresh yeast culture on it. Mark a "B" on the other and put a drop of dead (boiled) yeast culture on it. Add a drop of congo red solution to each slide and cover them with coverslips.

Observe the slides under the microscope. You might notice that the boiled yeast cells are clumped together. Clumping of boiled yeast cells often occurs and will not interfere with the experiment.

Yeast cells have no means of locomotion. If you observe any movement of the cells, it is due to the motion of the surrounding water.

1. Did you see the congo red inside the dead yeast cells? If you did, how did it enter? (Assume that boiling the yeast cells did not break the cell membrane.)

2. Did you see congo red inside the live cells? If you did not see the congo red, does this mean that it did not enter the live cells? Why or why not?

3. Based on your observations of the yeast cells, explain how and why the congo red moved, or did not move, across the cell membranes.

B. Water Concentration in Cells

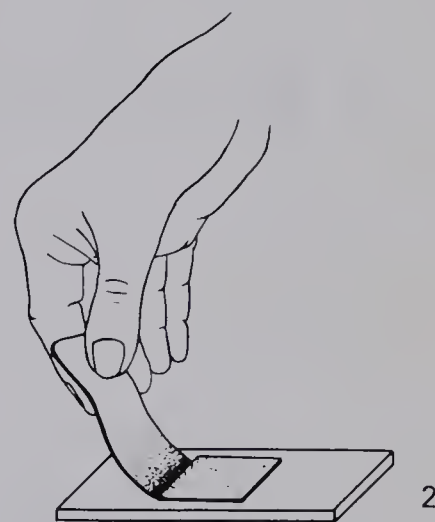
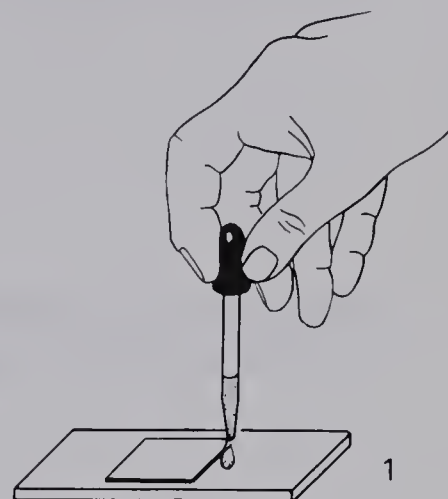
Place a young *Elodea* leaf in a drop of water on a clean slide and cover with a coverslip. Observe the leaf under high power with the compound microscope. Note that the cytoplasm containing the chloroplasts is in close contact with the cell wall.

Name _____ Date _____

4. Draw one *Elodea* cell.

Replace the water under the coverslip with 20 percent salt solution. Put a drop of 20 percent salt solution on the slide next to the coverslip. Touch a paper towel to the other side of the coverslip to draw the salt solution under the coverslip.

5. Draw one *Elodea* cell in the salt solution.



6. What is the effect of the salt solution on *Elodea* cells? Why did this happen?

7. What term is used to describe the *Elodea* cells in the salt solution?

ANALYSIS

8. What type of transport moves congo red into yeast cells?
-
-
9. Based upon your observations, what conclusion can you make about the difference between living and dead yeast cells?
-
-
-
10. What do you think fills the space between the cell membrane and the cell wall of a plasmolyzed *Elodea* cell?
-
-
-
11. Why would a lawn flooded with sea water die?
-
-
-
12. Herbaceous plants (plants without woody tissue) wilt when they need water. Why?
-
-

30 Water Movement in Plants

PURPOSE

To observe transpiration and the movement of water through plants.

MATERIALS

2 fresh celery stalks—1 with leaves and 1 without	2 beakers
geranium or other broad-leaved plant	self-sealing plastic wrap
iodine indicator stain	scalpel or razor blade
2 pieces of cobalt chloride paper	vaseline
	1 paper clip

INTRODUCTION

Transpiration is the process by which water evaporates from the parts of plants exposed to air, especially the leaves. One hectare of plants may transpire over 3 million litres of water in one growing season. This may seem undesirable, but it actually is of benefit to the plants.

In the first part of this lab you will test leaves to see whether transpiration is occurring. In the second part you will observe the movement of water through plants.

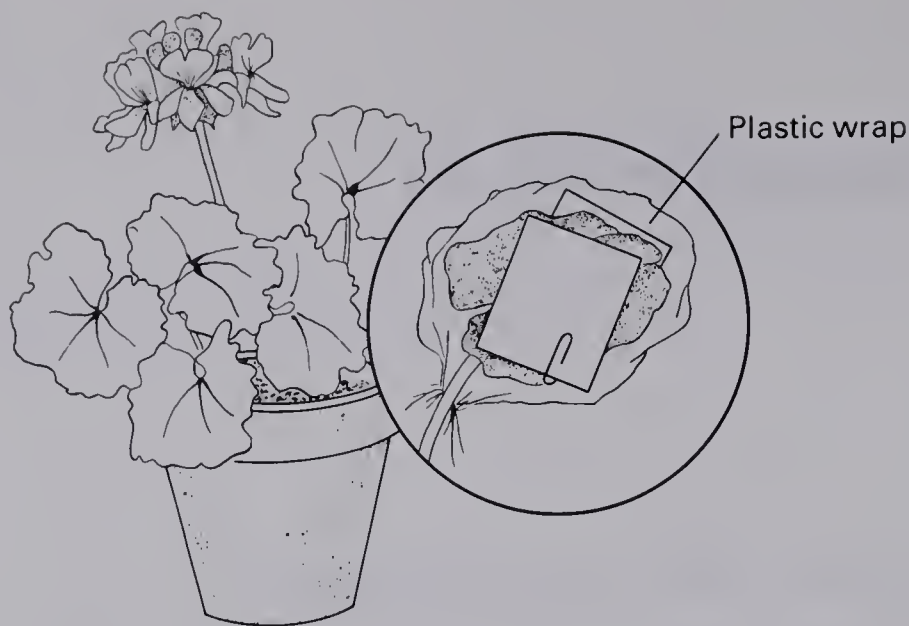
PROCEDURE

A. Transpiration

Obtain two pieces of cobalt chloride paper, which is used to detect the presence of water. If the paper is absolutely dry, it is blue. In the presence of even small amounts of moisture, the paper turns pink.

With a paper clip, attach a piece of cobalt chloride paper on top of a geranium leaf that is attached to the plant. Attach a second piece of cobalt chloride paper on the underside of the same leaf. Wrap the leaf and the papers with plastic wrap, taking care not to crush them. Press together the edges of the plastic wrap to seal off the leaf from the air. (See illustration on next page.)

Take Care: Be sure that your hands are dry. Dry the geranium leaf with a paper towel. If you get the cobalt chloride paper wet, the results of the experiment will be invalid.



Be sure that the plant's soil is very moist. Add water if necessary.

Every five minutes for fifteen minutes, check to see whether the papers have turned pink. Record your results on the data chart. Use a "P" to indicate a pink color and a "B" to indicate blue.

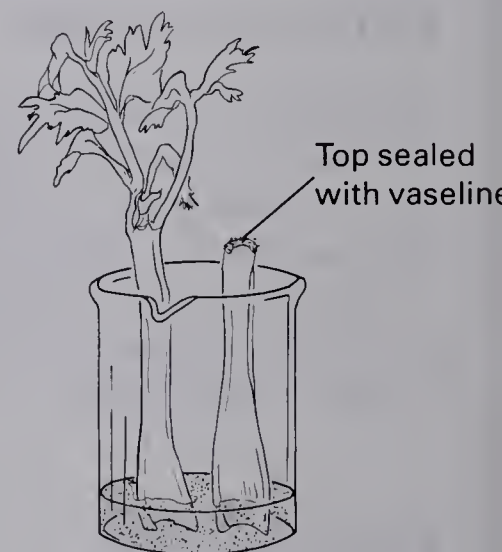
	<i>Time (in minutes)</i>			
	<i>0</i>	<i>5</i>	<i>10</i>	<i>15</i>
Paper on top of leaf	B			
Paper on underside of leaf	B			

B. Water Movement in Plants

Obtain two stalks of celery that are about the same length. One stalk should have leaves and the other should not have leaves. Cut the ends (opposite from the leaf end) off both stalks. Apply vaseline to the top of the stalk where the leaves were cut off—be sure the vaseline totally covers the top.

Fill one beaker with iodine solution to a depth of about 5 mm. Place the celery stalks, cut-ends-down, into the beaker. Put the beaker in sunlight or near a strong light. Fan or blow on the leaves periodically for five minutes, remove the stalks and rinse them with clean water. Observe the cut ends.

Two celery stalks in beaker with red food coloring



1. Is the brown color uniform over the cut ends or is it darker in certain places?

2. The part of the stalk that moves water throughout the plant is the xylem. Can you identify the xylem on the cut ends? How?

Next, starting at the bottom of each stalk, cut off 1-cm pieces. Determine how far the solution went up each stalk by observing the end of each cut section. Stop cutting when you reach a portion that was not penetrated by the brown coloring.

3. How far did the solution go in the stalk with leaves?

4. How far did the solution go in the stalk without leaves?

5. Draw the celery stem as it appears in cross section. Label the xylem.

ANALYSIS

6. Refer to the data chart in part A. Did the paper turn pink first on the top or the underside of the leaf? What does this result indicate about the rate of transpiration on the underside as compared to the top of the leaf?

7. In transpiration from leaves, water evaporates through the stomates. Do you think there are more stomates on the top or the underside of the leaf you tested? Why?

8. In part B, could transpiration have occurred in the stalk with leaves, without leaves, or both? Why?

9. Is transpiration of any benefit to the plant? If so, how?

31 The Human Heart Rate

PURPOSE

To study changes in the rate of the human heartbeat.

MATERIALS

stopwatch or wristwatch that shows seconds

INTRODUCTION

Each beat of the heart sends blood pulsing through arteries, veins, and capillaries. Day and night, the heart's rhythmic contractions propel blood to all parts of the body. The heart keeps the blood circulating to deliver oxygen and food to the cells and to remove wastes. If circulation stops for even a short time, the cells cannot survive.

When body movement increases, the cells use more energy. To accommodate this change, the heart rate must increase to pump enough blood to the cells. When body movement decreases, the heart rate decreases.

Regular vigorous exercise will enlarge the heart muscle. A larger heart works more efficiently than a smaller heart because it holds more blood and its muscle is stronger. Therefore, the larger heart takes fewer heartbeats to circulate the blood through the body.

The heart is made up of cardiac muscle, which has the power to contract all by itself—even if the heart is removed from the body. The autonomic nerves that are attached to the heart speed up or slow down the cardiac muscle's rhythmic beat.

There are two phases of a heartbeat. The contraction phase is called systole and the relaxation phase is called diastole. The sound of the heartbeat is actually the heart valves flapping as they close.

Do you know how fast your heart beats when you are resting? When you are active? How much blood do you think your heart pumps in one hour? After doing this lab, you should be able to answer these questions.

PROCEDURE

You are going to count your pulse at rest and during periods of activity. To count your pulse, do the following: Turn one hand palm up. Place the second and third fingers of your other hand on your wrist, below the thumb. You should be able to feel the blood pulsing through an artery with each beat of the heart.

A. Resting Heart Rate

Sit quietly and relax for at least two minutes before you begin.



Keeping time with a wristwatch, clock, or stopwatch, count your pulse for exactly twenty seconds. Repeat this procedure two more times. Find your average count for twenty seconds, then multiply the average number by three to determine your pulse rate for one minute.

Record your rate on the chalkboard in a numbered box of the “at rest” table. Remember your box number for use in the other tables. If you are in an athletic training program such as track or swimming, put an asterisk (*) after your pulse rate on the table.

Record all the class data from the chalkboard on Table 1.

Table 1. Heart Rate at Rest

<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>
1		7		13		19		25	
2		8		14		20		26	
3		9		15		21		27	
4		10		16		22		28	
5		11		17		23		29	
6		12		18		24		30	

B. Heart Rate After Mild Exercise

Engage in some form of mild exercise for three minutes. You may walk around the room, or do side bends, body twists, or other light exercise as directed by your teacher.

After exercising, take three pulse counts of twenty seconds each. Determine your pulse rate as you did in part A and record the rate on the “mild exercise” table on the chalkboard. Use the box number you had in part A and add an asterisk if you are in training.

Record all the class data on Table 2.

Table 2. Heart Rate After Mild Exercise

<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>	<i>Box</i>	<i>Rate</i>
1		7		13		19		25	
2		8		14		20		26	
3		9		15		21		27	
4		10		16		22		28	
5		11		17		23		29	
6		12		18		24		30	

C. Heart Rate After Vigorous Exercise

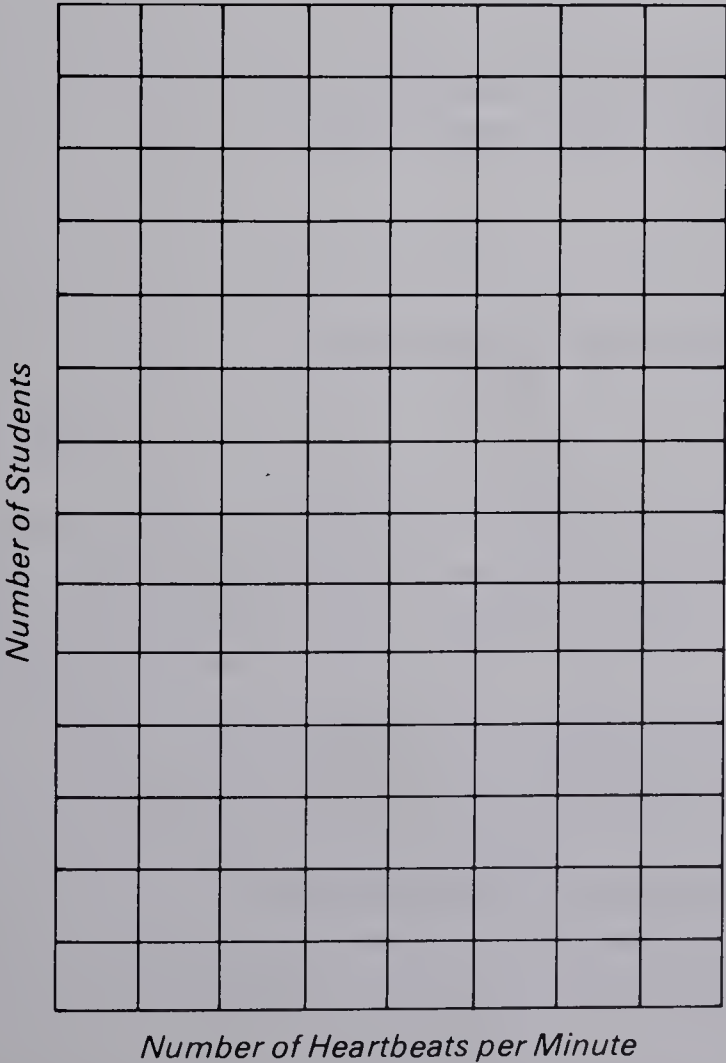
Do some form of vigorous exercise for three minutes. The exercise can be running in place, jumping jacks, push-ups, sit-ups, or similar exercise. Determine your pulse rate and record it in the “vigorous exercise” table on the chalkboard. Use the same box number as before and add an asterisk if you are in training.

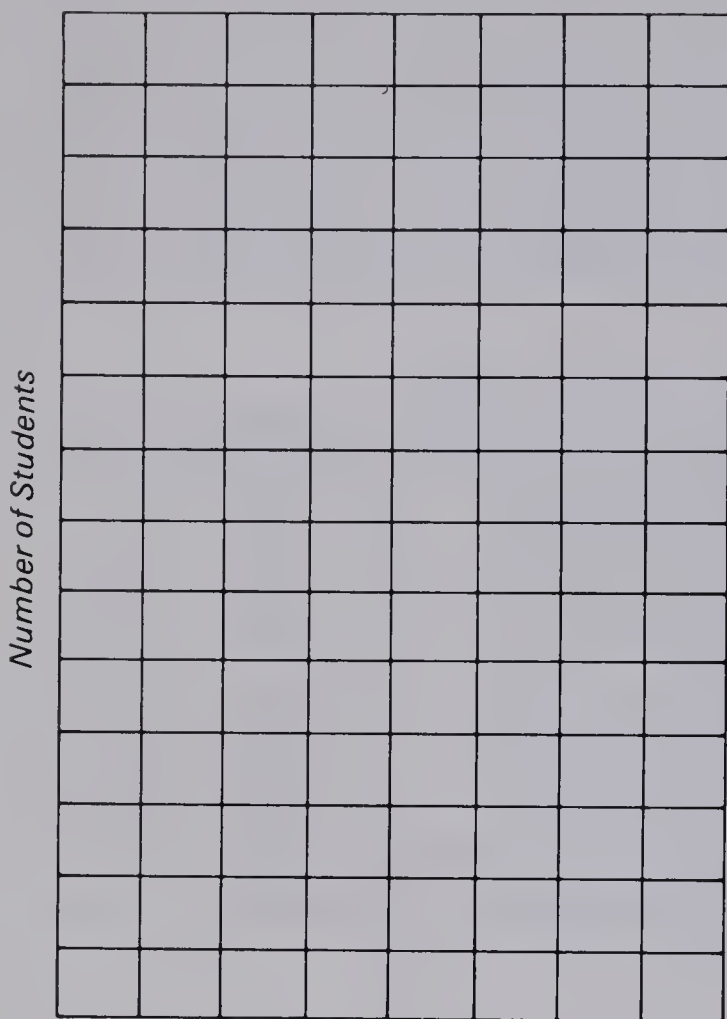
Record all the class data on Table 3.

Table 3. Heart Rate After Vigorous Exercise

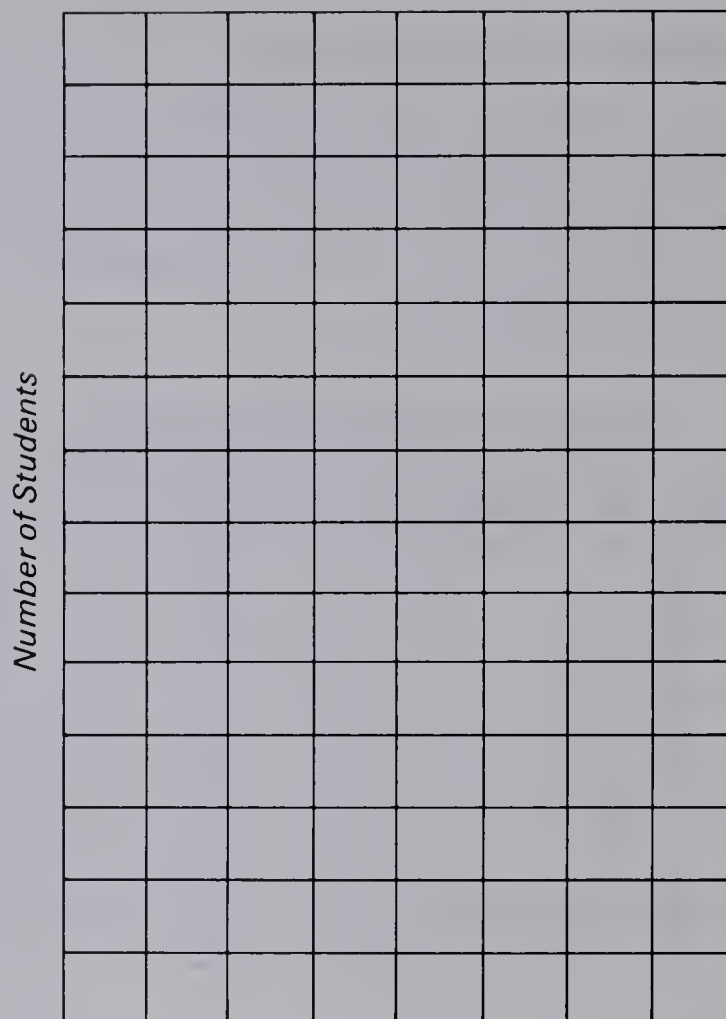
Box	Rate	Box	Rate	Box	Rate	Box	Rate	Box	Rate
1		7		13		19		25	
2		8		14		20		26	
3		9		15		21		27	
4		10		16		22		28	
5		11		17		23		29	
6		12		18		24		30	

Prepare histograms on the graphs provided for each table of data. If you need help making the histograms, see the Graphing lab. When a piece of data from the table has an asterisk, put an asterisk next to the *X* on the graph representing that piece of data.





Number of Heartbeats per Minute



Number of Heartbeats per Minute

ANALYSIS

1. Do the people in an athletic training program (with asterisks) tend to be concentrated at the low, middle, or high range of each histogram?

2. If they are concentrated in a certain range, how do you account for it?

3. Note the total range of data on each histogram. On which graph is the range the greatest?

4. Calculate the percent increase in your heart rate from rest to mild exercise. Use the following formula:

$$\frac{(\text{rate after mild exercise} - \text{rate at rest})}{\text{rate at rest}} \times 100 = \% \text{ increase in rate}$$

5. Calculate the percent increase in your heart rate from rest to vigorous exercise.

6. The average heart pumps about 80 mL of blood in each contraction. Calculate the volume of blood your heart pumps in one minute when you are resting, and then determine how much blood would be pumped in one hour at this rate.

7. Make the same calculations using the data for vigorous exercise.

FOLLOW-UP

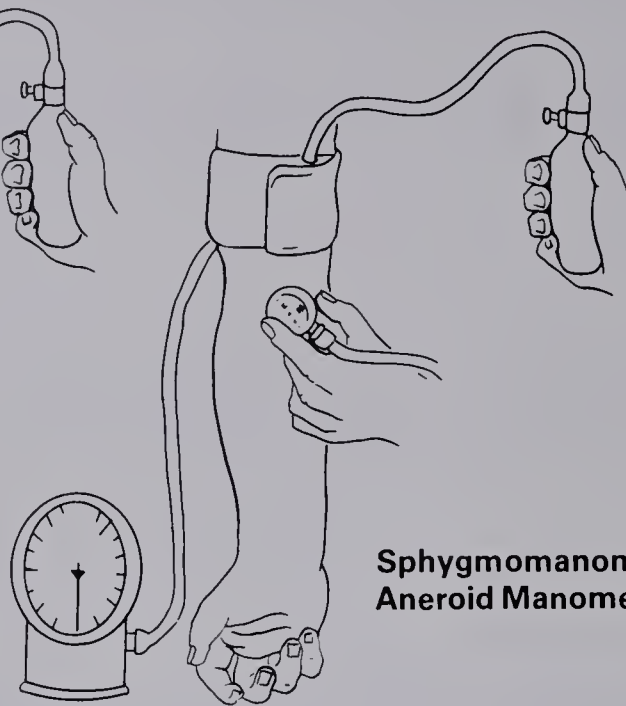
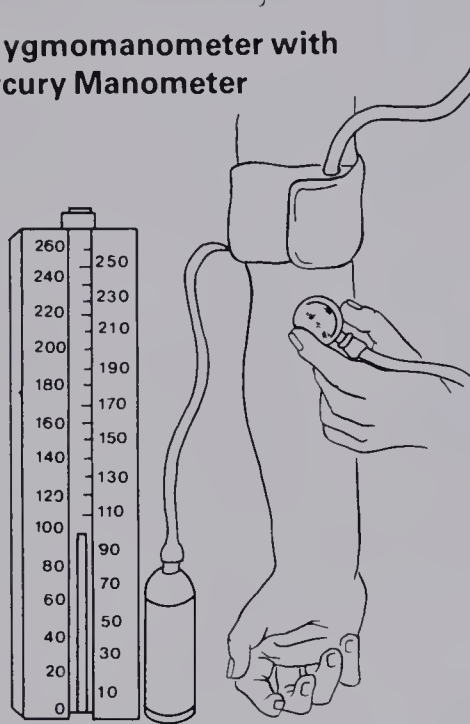
- Blood exerts pressure against the walls of the arteries as it is pumped through the body. When the heart contracts, the pressure is called systolic pressure. When the heart relaxes, the pressure is called diastolic pressure.

Blood pressure is measured with a sphygmomanometer and a stethoscope. The sphygmomanometer indicates blood pressure in millimetres of mercury. If your school has the equipment, work in pairs and take each other's blood pressure.

Wrap the cuff of the sphygmomanometer around your partner's upper arm; the arm must be bare under the cuff. Place the stethoscope disk over the artery just below the cuff. Listen through the stethoscope for your partner's heartbeat.

Turn the valve below the rubber ball on the sphygmomanometer clockwise until the valve is closed. Squeeze the ball to pump air into the cuff until the heartbeat sound stops. The pressure of the cuff against the artery stops the flow of blood.

Sphygmomanometer with Mercury Manometer



Sphygmomanometer with Aneroid Manometer

Slowly open the valve to let air out of the cuff. As you release the air, watch the manometer. Note the reading when you hear the first heartbeat—this is the systolic pressure.

Continue to release the air, very slowly. The heartbeats will become muffled. When you no longer hear any sound, note the reading on the manometer. This is the diastolic pressure.

Blood pressure readings vary according to many factors, including age and health. The normal range for people aged 14 to 18 years is 100-140 systolic and 50-70 diastolic. The reading is noted as a fraction with systolic pressure as numerator and diastolic pressure as denominator—for example, 110/60.

- Use the stethoscope to listen to your heartbeats. Place the disk flat against your chest, about 8 cm to the left of the bottom of the breastbone. The sound you will hear is the closing of the heart valves.

32 The Kidney

PURPOSE

To become familiar with the structure and the function of the kidney.

MATERIALS

preserved, double-injected sheep
or pig kidney

dissecting pan lined with
paper towels

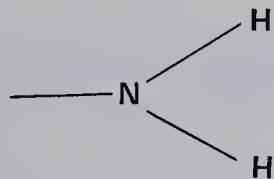
scalpel

INTRODUCTION

One of the most important organs in vertebrates is the kidney. The kidneys, which occur in pairs, help maintain the correct balance of substances in the blood. They do this by filtering blood to remove wastes and other substances.

Among the most important substances that the kidneys remove from the blood are nitrogenous (nitrogen-containing) wastes. Most nitrogenous wastes are produced in the liver.

The liver breaks down amino acids, which contain nitrogen, to obtain energy. This process also produces amino groups as byproducts. An amino group is made up of one atom of nitrogen and two atoms of hydrogen.



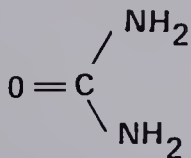
An amino group

The liver converts amino groups into nitrogenous wastes in two steps. First, the amino groups are converted into ammonia, which is very poisonous. Then, the liver converts most of the ammonia into urea, which is less poisonous than ammonia. A small amount of the ammonia is converted into uric acid, which is even less poisonous than urea.

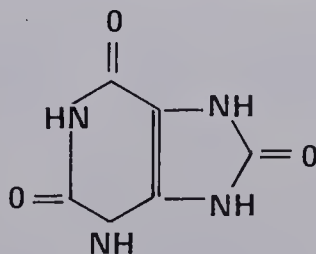
Each molecule of urea contains the amino groups from two ammonia molecules and a portion of a carbon dioxide molecule. Each uric acid molecule is made of four ammonia molecules and three carbon dioxide molecules.



Ammonia



Urea



Uric Acid

The liver dumps the urea and uric acid that it makes into the blood. The kidneys filter these wastes from the blood and produce urine.

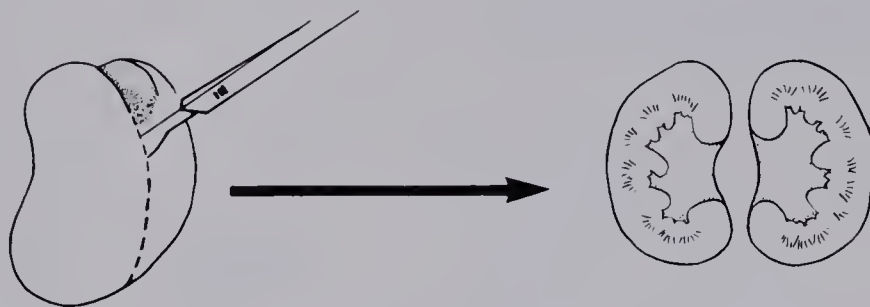
In this lab you will examine a kidney and learn how it filters substances.

PROCEDURE

A. Anatomy of the Kidney

Place a kidney in a dissecting pan lined with wet paper towels. With a sharp scalpel, cut the kidney in half longitudinally (lengthwise), traveling downward along the edge of the kidney.

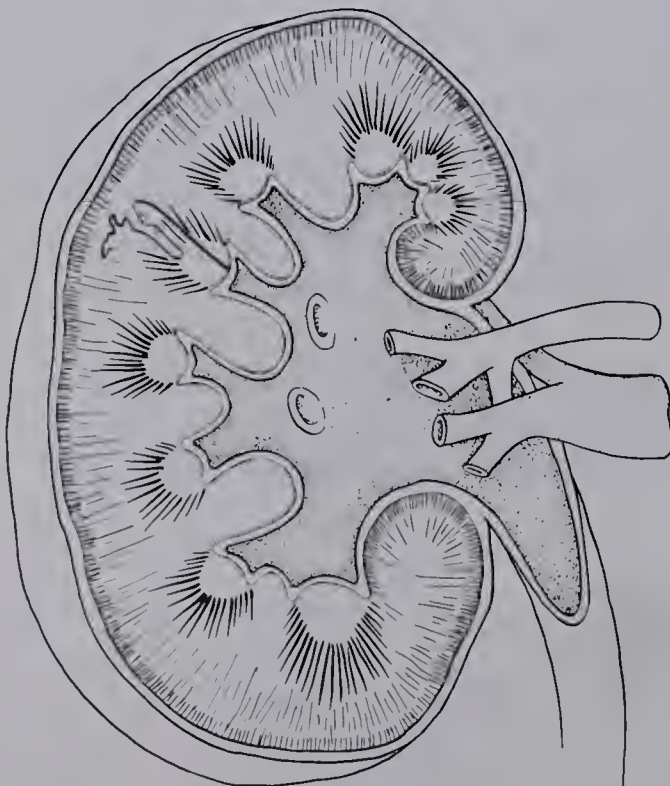
Caution: Cut in a direction away from your fingers.



Examine the cut surface of the kidney. The outer cortex contains thousands of filtering structures called nephrons. The inner layer, called the medulla, contains urine ducts. These ducts empty into the funnel-shaped renal pelvis, the kidney's urine-collecting area. The ureter, the tube leading out of the renal pelvis, transports the urine to the urinary bladder.

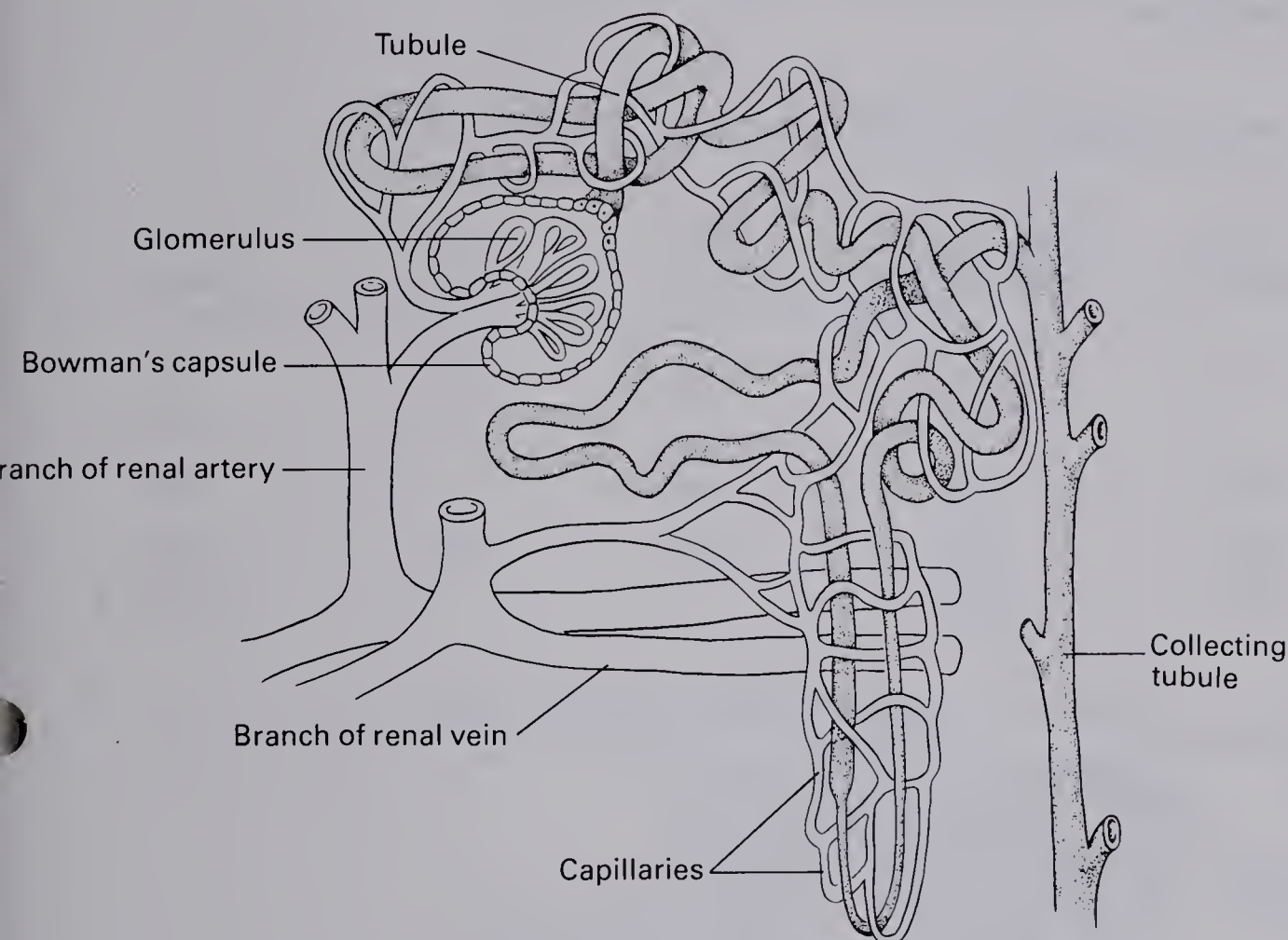
The renal artery brings blood into the kidney to be filtered. It is colored red by latex injected when the kidney was preserved. The renal vein, colored blue, channels blood from the kidney to the heart, where it is recirculated.

1. On the diagram of the kidney, label the cortex, nephron, medulla, renal pelvis, ureter, renal artery, and renal vein.



B. Nephron Structure and Function

The amounts of dissolved substances in the blood must remain at fairly constant levels in order for the body to function properly. The function of nephrons in the kidney is to remove wastes, as well as excess amounts of certain dissolved substances.



A nephron is a twisted tube with a round structure—Bowman's capsule—at one end. The Bowman's capsule is a membrane that filters fluids from blood.

Fluid from the renal artery enters Bowman's capsule through a network of capillaries called the glomerulus. Certain fluids (such as water) and dissolved substances pass from the glomerulus through Bowman's capsule into the tubule of the nephron. The fluid in the tubule is called the filtrate.

The blood remaining in the glomerulus flows into capillaries surrounding the tubule. As the filtrate flows through the tubule, certain substances, including glucose and water, move from the filtrate back into the capillaries. This movement occurs by both active and passive transport. Other substances are actively secreted from the capillaries into the tubule.

The substances that remain in the filtrate are wastes—urea, uric acid, salts, ions, and excess water. Collectively, these substances make up urine. The urine flows into the collecting tubule, and from there into the renal pelvis.

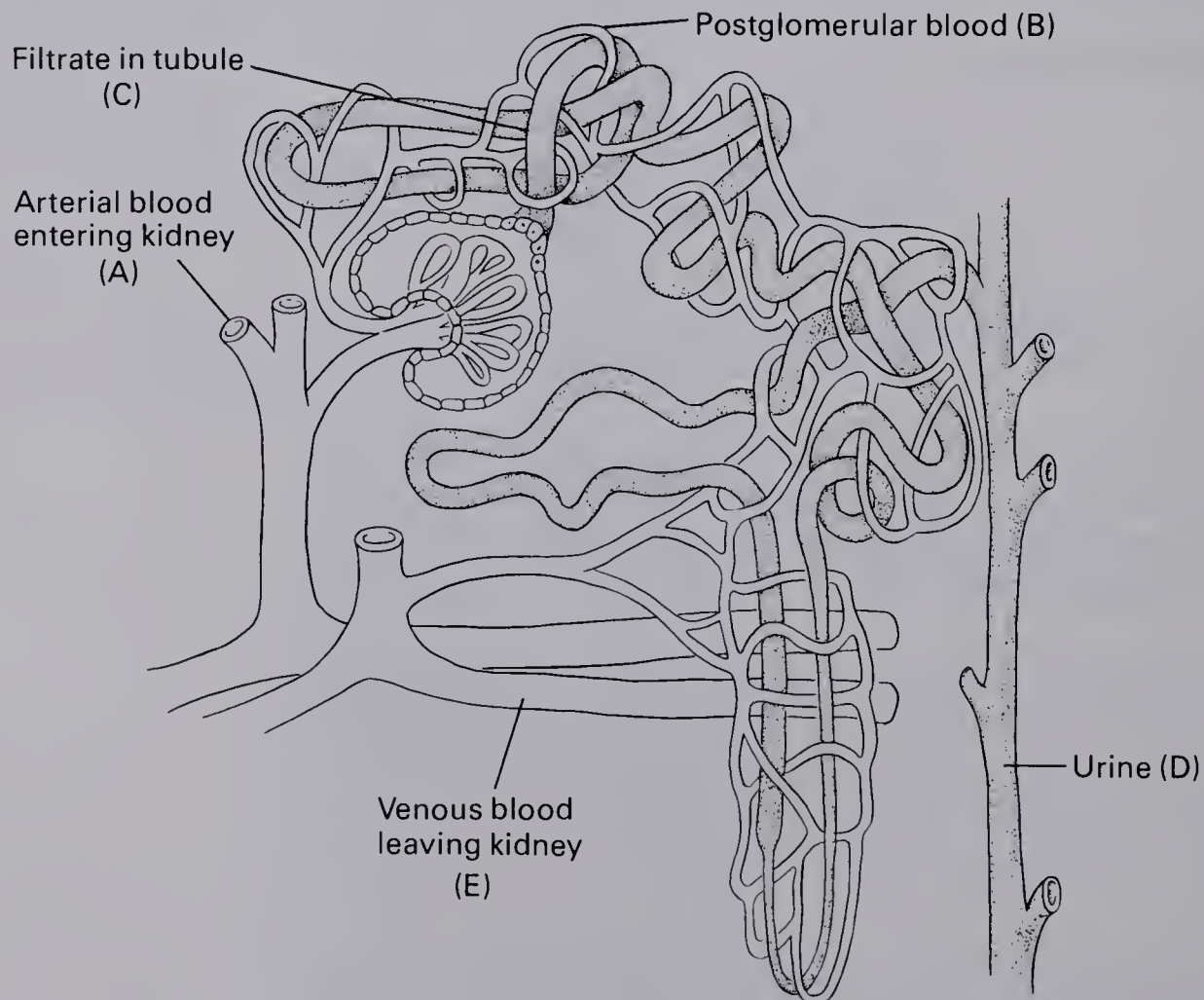
In humans, about 600 L of blood flow into the kidneys in one day. About 100 L of this fluid filter through Bowman's capsule and become filtrate. Only 1.5 L become urine and are excreted from the body.

The chart below shows how the concentration of five substances dissolved in the blood change as the substances pass through the kidney.

Concentration is expressed as the ratio of the number of dissolved particles, or solute, to the number of molecules of liquid, or solvent. For example, when you make instant lemonade, the solute is the lemon crystals and the solvent is water. To change the concentration of lemonade, you can vary the amount of either solute or solvent. To make the lemonade stronger, either add more lemon crystals or remove some of the water by letting it evaporate.

Concentrations of Dissolved Substances (mg/100 mL of fluid)

<i>Dissolved Substances</i>	<i>Arterial Blood (A)</i>	<i>Postglomerular Blood (B)</i>	<i>Filtrate (C)</i>	<i>Urine (D)</i>	<i>Venous Blood (E)</i>
Urea	30	30	30	1800	15
Uric Acid	4	4	4	50	3.6
Glucose	100	100	100	0	98
Salts	900	900	900	2300	850
Proteins	8000	9000	0	0	8020



- According to the chart, what substances pass from the glomerulus through Bowman's capsule into the tubule to become filtrate?

3. Urea, uric acid, glucose, and salts show no change in concentration in columns A, B, and C. Why not?

4. How do you account for the increase in the protein concentration from the arterial blood to the postglomerular blood?

5. Why did the protein concentration decrease from the postglomerular blood to the venous blood?

6. How do you account for the increases in concentration of urea, uric acid, and salts in the urine?

7. What do you think happened to the glucose in the filtrate?

8. Why was the glucose concentration lower in the venous blood than in the postglomerular blood?

9. Which *one* of the dissolved substances is removed from the blood most completely through this system?

ANALYSIS

10. Where are urea and uric acid formed?

11. Briefly describe the function of the following:

Cortex: _____

Nephrons: _____

Medulla: _____

Renal pelvis: _____

Ureter: _____

Renal artery: _____

Renal vein: _____

12. What part of the nephron actually filters the blood?

13. What is the filtrate composed of?

14. How are substances the body needs reabsorbed into the blood from the filtrate?

15. How do the kidneys maintain the correct balance of substances in the blood?

33 Fish Anatomy

PURPOSE

To become familiar with the anatomy of a fish.

MATERIALS

preserved perch	dissecting probe
dissective microscope	forceps
slide	scissors
dissecting pan with wax	plastic food bag
dissecting pins	

INTRODUCTION

Fish are coldblooded aquatic vertebrates whose streamlined bodies aid in swimming. They are characterized by having fins for swimming, gills for breathing, and hearts with only two chambers.

The skeleton of some fish, such as sharks, is made of cartilage. This is the same tough material that gives shape to the human nose and ears. In more advanced types of fish, the skeleton is made of bone. Bony fish are covered with scales, which help water flow smoothly over their bodies.

In this lab, you will dissect a bony fish.

PROCEDURE

A. External Anatomy

Place a preserved perch in a dissecting pan lined with wet paper towels. Examine the head region. On each side of the mouth is a semicircular flap called the **operculum**, which covers the gills. Water enters the mouth, flows over the gills, and leaves through the opening covered by the operculum. The fish breathes by absorbing oxygen dissolved in the water through its gills.

Locate the fish's nostrils. Inside the nostrils are **olfactory organs**, which detect chemical substances dissolved in the water. Insert a probe into one of the fish's nostrils. Open the mouth to see if the probe comes into the mouth.

1. Does the nostril lead into the mouth?

2. Do the nostrils play any role in the fish's breathing? Why or why not?

3. How could the nostrils aid the fish in smelling?

Examine the fish's six types of fin. On its dorsal (back) side is the spiny anterior dorsal fin and the soft posterior dorsal fin. On its tail is the caudal fin. On its ventral (under) side is the anal fin, near the anus, and the pelvic fin. Just behind the fish's head is the pectoral fin.

4. In each of the boxes below, draw one of the fish's six types of fin. Draw the anterior dorsal fin in box 1 and proceed clockwise around the fish. Label each fin.

5. Which of the fins are paired (identical fins on each side of the body)?

6. Fish use the dorsal and anal fins for stability and to stay upright. Based on their structure and position, what do you think the other fins are used for?

Find the fish's lateral line, a series of grooves along its skin that run nearly the length of the fish. Cells in the lateral line are sensitive to vibrations in the water. This enables the fish to tell if another animal is moving through the water.

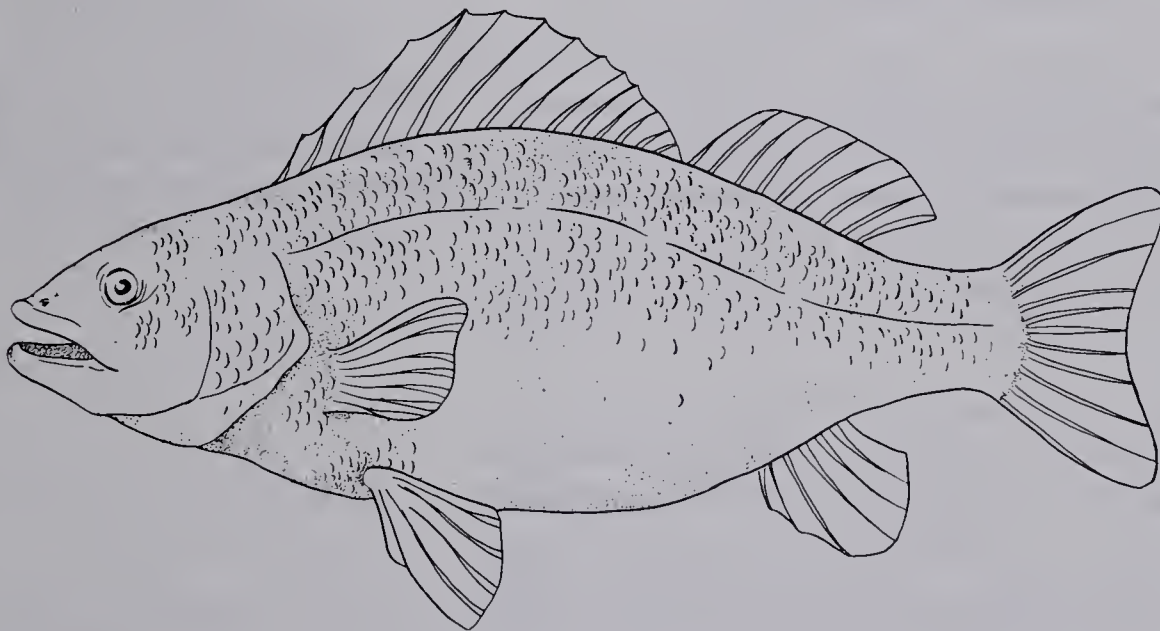
Use forceps to remove a scale from the fish. Put the scale on a slide and observe it under a dissecting microscope.

The concentric rings are lines of growth. As the fish grows, the scales grow larger. Because the coldblooded fish grows slowly at low temperatures, the growth lines are formed close together during winter. Each winter's growth lines appear as a ring on the scale. So, you can approximate the age of the fish by counting the rings.

7. Draw the fish scale, showing the growth lines.

8. About how old is the fish?

9. On the outline drawing of a perch, label the nostril, operculum, lateral line, and fins.



B. Internal Anatomy

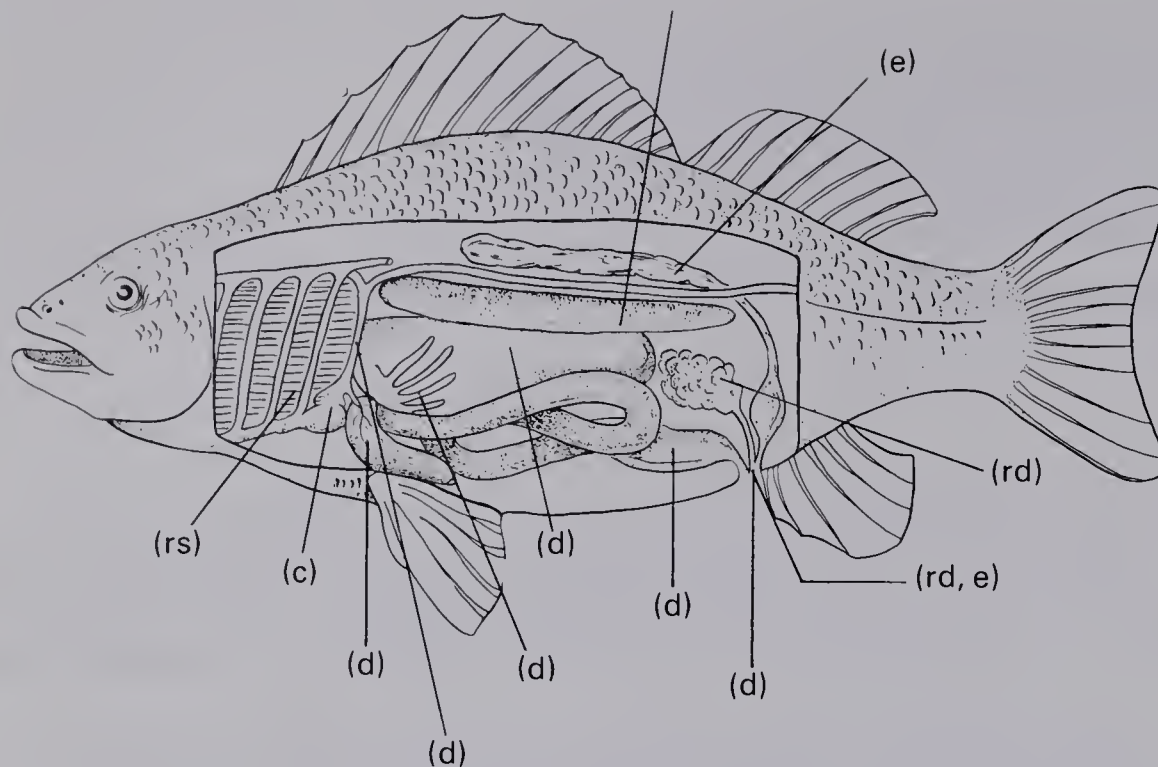
Respiratory System Using scissors, cut the operculum off of one side of the fish to expose the gills. Each gill consists of feathery filaments attached to a gill arch.

10. How many gills do you find?

Remove a portion of one gill by cutting it with scissors at its point of attachment to the arch. Examine the feathery structure.

11. How does the feathery structure of the gills aid in gas exchange?

12. On the fish diagram, label the gills.



System to which Part Belongs

- | | |
|------------------------|--------------------------|
| (c) circulatory system | (rs) respiratory system |
| (d) digestive system | (rd) reproductive system |
| (e) excretory system | |

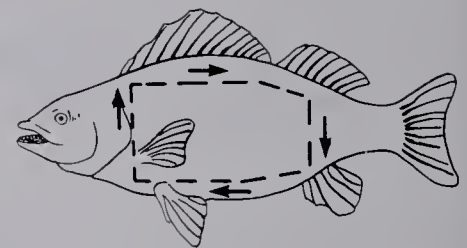
To expose the fish's internal organs, you will cut out a section of the muscular body wall. With sharp scissors, make an incision close to the anus. Cut forward to the gills (where you removed the operculum). From the top of the gill area, cut along the body to a point above your first incision. Cut downward to the incision. Carefully remove the flap of body wall, using a scalpel if necessary.

Circulatory System The fish's two-chambered heart lies ventral to, just behind, the gills. Veins carry blood to the upper chamber, the atrium. The blood then flows into the larger chamber, the ventricle. Ventricle muscles pump the blood through arteries to the gills, where it exchanges carbon dioxide for oxygen. The arteries then channel blood, carrying food absorbed from the intestine and oxygen, throughout the body.

13. Examine the heart. Describe the difference in the muscular nature of the two chambers. Why do you think one chamber is more muscular than the other?

14. On the fish diagram, label the heart.

Digestive System Food enters the digestive tract through the fish's mouth. It passes through the throatlike pharynx into the esophagus,



Take Care: Cut carefully to avoid destroying the organs beneath the body wall.

the tube that leads to the stomach. The stomach's capacity is increased by several pouchlike structures called pyloric caeca. After being partially digested in the stomach, the food enters the winding intestine. Digestion is completed there, with the aid of the bean-shaped liver. Undigested food is removed through the anus.

Use your probe to trace the digestive tract, starting at the esophagus. You may have to push aside the liver and gills to see the esophagus.

15. How do you think the pyloric caeca aid the stomach in digestion?

16. On the fish diagram, label the esophagus, stomach, pyloric caeca, intestine, liver, and anus.

Excretory System Lying just beneath the spine are the kidneys, which appear as dark masses of tissue. The kidneys absorb waste products from the blood. The waste is excreted as urine through the urogenital opening, just behind the anus.

17. On the fish diagram, label the kidneys and urogenital opening.

Reproductive System The fish's reproductive organ, or gonad, is located above the intestine and leads into the urogenital opening. In a female fish, the organ is a large yellow mass of tissue called the ovary. In a male, the organ is a smaller, whitish mass of tissue called the testis.

18. Is your fish a male or a female?

19. On the fish diagram, label the gonad as either ovary or testis, depending on the sex of your fish.

The Air Bladder Between the gonad and the kidneys is a sac called the air bladder. The fish uses the air bladder to regulate its position in the water. So, it plays an important part in the fish's ability to live and swim in the water.

The fish inflates the air bladder with gases produced in the blood. As the amount of gas in the bladder changes, the fish's vertical position in the water changes.

20. If the amount of gas in the bladder increases, what do you think happens to the fish's position?

21. What function does the air bladder perform?

22. On the fish diagram, label the air bladder.

When you have finished your dissection, wrap the fish in the paper towels and dispose of it as instructed by your teacher. If time remains in your class period, you might wish to perform the follow-up dissection before disposing of the fish.

ANALYSIS

23. Write the system (or systems) to which each structure listed below belongs. (Systems: respiratory, circulatory, digestive, excretory, reproductive.)

anus _____	mouth _____
arteries _____	ovary _____
esophagus _____	pharynx _____
gills _____	pyloric caeca _____
heart _____	stomach _____
intestine _____	testis _____
kidneys _____	urogenital opening _____
liver _____	veins _____

24. The fish's mouth and pharynx are wide and its esophagus is elastic. What does the nature of these structures indicate about the fish's feeding?

25. Some fish, such as sharks and rays, do not have air bladders. How must they maintain their vertical position in the water?

FOLLOW-UP

Using scissors, cut away the body wall between the fish's eyes until you reach the skull. With a scalpel or razor blade, carefully scrape away the top portion of the skull. This should expose the brain and anterior portion of the spinal cord. These organs are part of the fish's nervous system. Draw the brain, showing the lobes.

34 Frog Anatomy

PURPOSE

To become familiar with the anatomy of a frog.

MATERIALS

preserved frog	dissecting probe
compound microscope	forceps
slides and coverslips	scissors
dissecting pan with wax	scalpel
dissecting pins	plastic food bag

INTRODUCTION

The vertebrates include fish, amphibians, reptiles, birds, and mammals. Amphibians, such as the frog, live in water during their immature years and live primarily on land during their adult years. The adult frog is a good example of the body organization of vertebrates that live on land.

PROCEDURE

A. External Anatomy

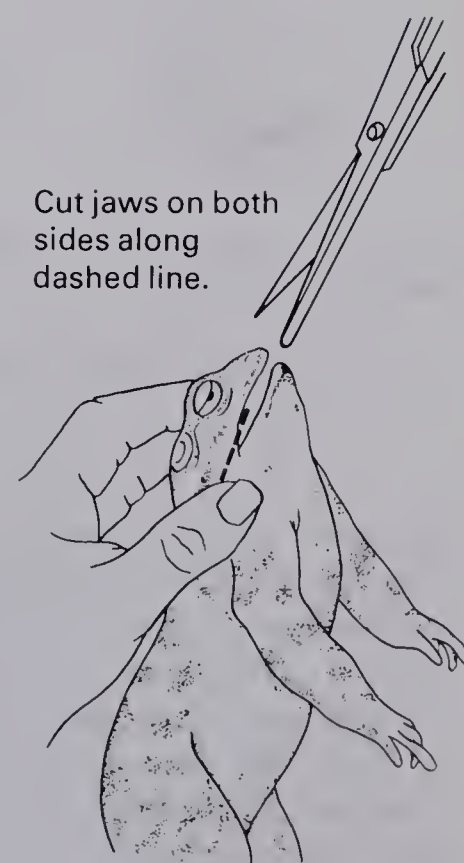
Line a dissecting pan with wet towels. Rinse a preserved frog with water to remove as much of the preservative as possible, and place the frog in the pan.

Skin Feel the skin of the frog. Note the absence of scales, hair, and feathers. The smooth, moist skin aids in respiration. Frogs obtain oxygen in a variety of ways: by absorbing oxygen through the skin, through the membrane covering the inside of the mouth, and through the lungs. While frogs are inactive, absorption through the skin meets their oxygen needs.

Head The two holes near the mouth end of the head are the external nares, the outer nose openings. Just behind the eyes are the ear drums, which are round, flattened areas in the skin.

To examine the interior of the mouth, use scissors to cut the edges of the mouth at each hinge joint. Open the mouth wide; if necessary, pry the jaws apart with a scalpel handle.

Rub your finger along the roof of the mouth. You will feel a row of



small teeth called the maxillary teeth. Below these are two sharp mounds called vomerine teeth.

Close to the teeth are two openings, the internal nares. Insert a probe into an internal nare.

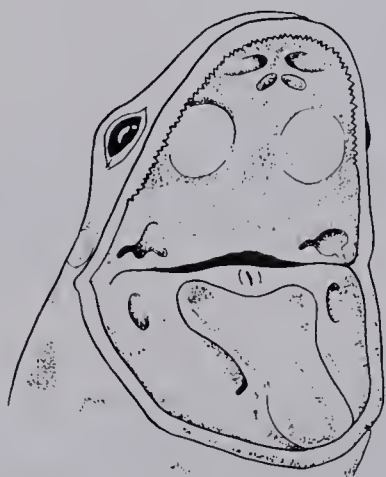
1. Where does the probe emerge?

The wide opening in the center of the mouth is the top of the *esophagus*, the tube that leads to the stomach. Below the esophagus is a vertical slit called the *glottis*, which leads to the lungs.

The frog's tongue fills most of the lower jaw. In living frogs the tongue is sticky.

2. Where is the tongue attached to the jaw? How would this place of attachment and the tongue's stickiness be useful to the frog?

-
-
3. On the diagram of the frog's mouth, label the maxillary teeth, vomerine teeth, internal nares, esophagus, glottis, and tongue.

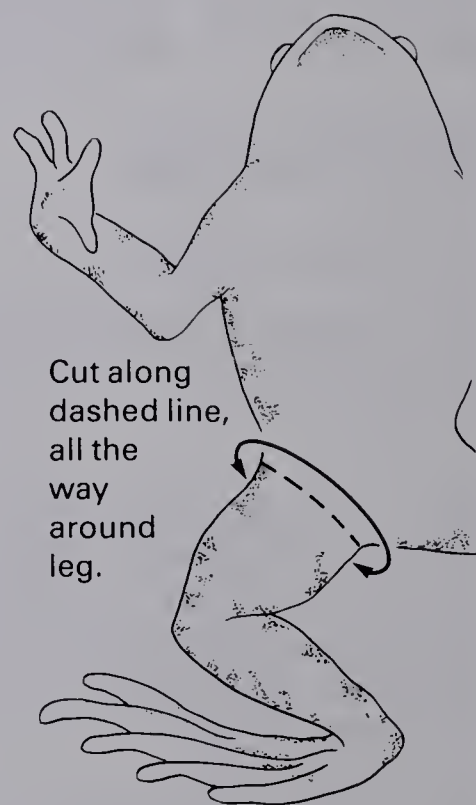


Limbs Examine the frog's front and hind limbs. Note the webbing between the toes.

4. What do you think is the function of the webbing?

Muscles With the point of sharp scissors, carefully make an incision through the skin where one of the hind limbs joins the body. Cut the skin around the top of the hind limb, as illustrated. Peel the skin off the leg to expose the skeletal muscles underneath. With forceps, remove the thin connective tissue covering the muscles.

Note that the muscles taper at the ends. The tapering ends are attached to the bones with tough white cords called tendons. The two points of attachment are called the origin and the insertion.



Take Care: The frog's skin is very thin. When dissecting skin, make careful, shallow cuts to avoid damaging the structures under the skin.

When the muscles contract, they cause the tendons to move the bones. The origin does not move much during contraction of the muscle. Most of the movement occurs at the insertion.

Locate the large gastrocnemius muscle on the lower back side (the calf) of the leg. The tendon that attaches it to the bone is the Achilles tendon. Note that at the upper end of the muscle there is more than one origin. Find the insertion at the foot.

5. What movement do you think would result from the contraction of the gastrocnemius muscle?
-

Dissect out the gastrocnemius muscle by cutting the tendon at the origin and insertion. Cut out a thin layer of muscle fiber, about one square centimetre, and place it on a slide. Shred the muscle with the probe. Add a drop of water and cover with a coverslip.

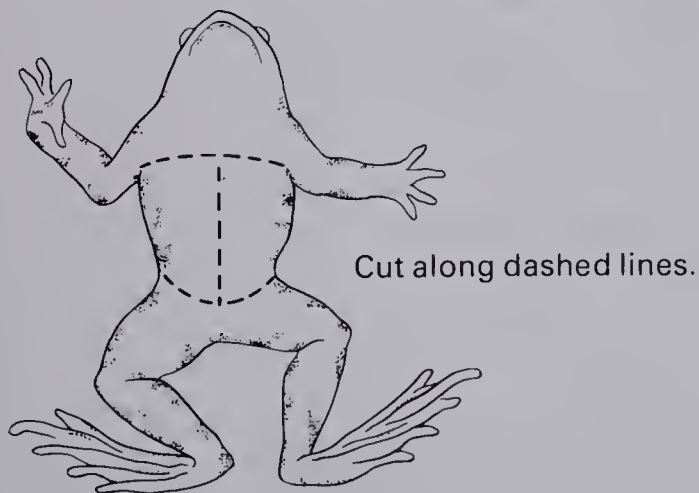
Observe the slide under a compound microscope. Note the alternate bands of light and dark lines, called striations. Muscles that have these bands are called striated muscles. Muscles that do not have striations are called smooth muscles.

6. Do the striations run along the length of the muscle or across the muscle?
-

B. Internal Anatomy

Place the frog in the pan with the ventral side facing up. Pin the limbs to the wax in the pan.

With forceps, pick up the loose skin just above the anal opening. Make an incision through the raised skin. Cut the skin along the center of the body to the base of the head. Cut laterally from the central cut to each of the limbs. Pin the skin flaps back from the body wall.



Now make the same cuts through the muscle of the body wall as you did through the skin. Raise the body wall with the scissors as you cut to avoid damaging the structure below. When you reach the forelimbs, you will have to cut through the sternum, the bone that connects the forelimbs. Pin back the muscle flaps to expose the internal organs.

In order to fully examine the internal organs, you will probably have to remove certain structures. A large mass of black and white eggs may fill much of the abdomen. If so, your frog is a female. Carefully remove the egg mass with forceps, making cuts where necessary.

There may also be several yellow fingerlike organs. These are fat bodies. The fat bodies store food, which is used during periods of inactivity. Carefully cut where necessary to remove the fat bodies.

Digestive System In the middle of the body cavity is the liver, the largest organ in the body. The reddish-brown liver consists of two large lobes with a smaller lobe between them. The liver produces bile, which aids in the digestion of fats. It also stores food in the form of glycogen and plays a role in breaking down poisonous wastes.

Carefully lift the liver to see the other organs of the digestive tract. On the underside of the liver is a greenish sac called the gallbladder. This stores the bile produced by the liver before it passes into the small intestine.

The oval whitish sac on your right-hand side is the stomach, where food is partially digested. At its top end, the stomach is connected to the esophagus, which channels food from the mouth. At its bottom end, the stomach narrows to a bulge called the pyloric valve. Run your finger over the valve—it feels like a knot. When this donut-shaped muscle contracts, food is prevented from leaving the stomach.

Lying just above the curved end of the stomach is the thin, ribbon-like pancreas, which secretes digestive enzymes into the small intestine.

The small intestine is the narrow tube leading away from the stomach. Digestion is completed in the small intestine, as is most nutrient absorption. The small intestine loops in tight coils down to the large intestine, a short wide tube. The large intestine leads to the cloaca, a large sac that passes wastes out of the body.

After you have examined the digestive system, carefully dissect out the liver. This will expose more of the internal organs.

7. On the frog diagram, label the liver, gallbladder, pancreas, stomach, pyloric valve, small intestine, large intestine, and cloaca.

Circulatory System The reddish triangular organ in the middle of the upper body is the heart. It has three chambers. The two upper chambers are the atria, which collect blood from the veins. Blood flows from the atria into the lower chamber, the ventricle. The muscular ventricle pumps blood throughout the body through the arteries.

Locate the small, red pea-shaped organ in the connective tissue near the small intestine. This is the spleen, which manufactures white blood cells and removes dead red cells from the blood.

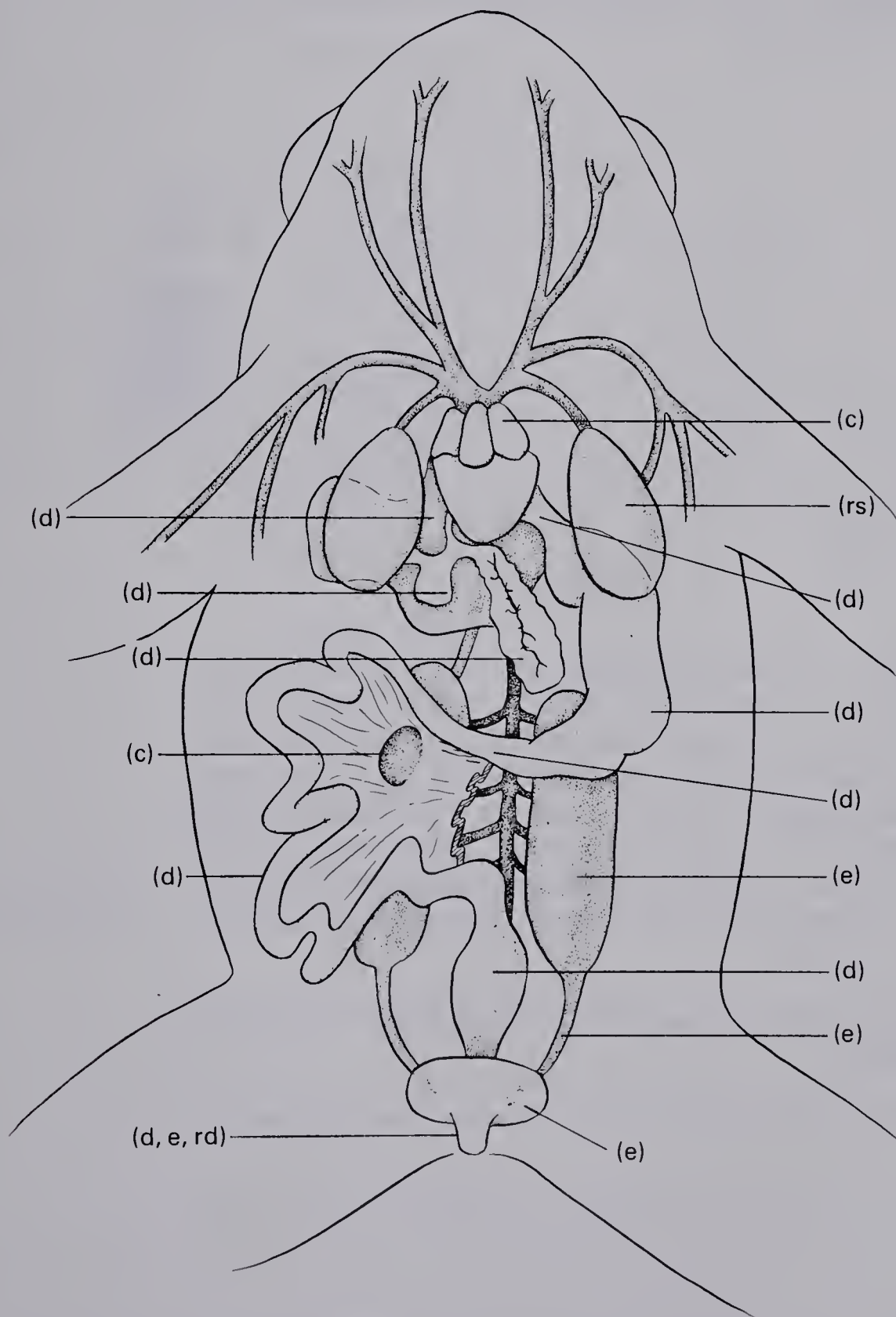
8. On the frog diagram, label the heart and spleen.

Respiratory System The lungs are two spongy elongated bags located on both sides of the heart. Frog lungs work much like balloons. The frog takes air into its mouth and enlarges the mouth cavity by extending the skin on the lower jaw. Then it closes the mouth and the nares, pushing the air through the glottis into the lungs.

9. On the frog diagram, label the lungs.

System to which Part Belongs

- (d) digestive system (e) excretory system
 (c) circulatory system (rd) reproductive system
 (rs) respiratory system

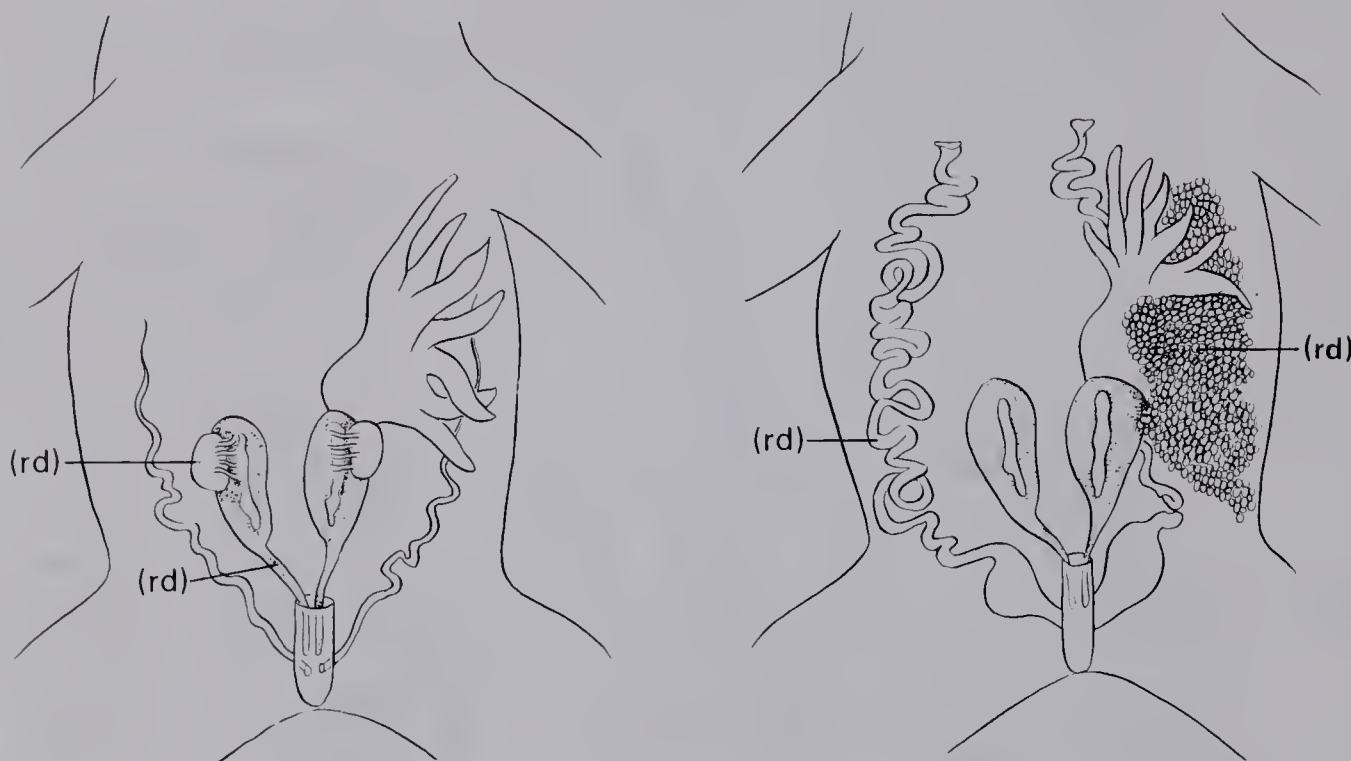


Reproductive System If your frog was filled with eggs, it is a female ready for breeding. If your frog is a female not ready for breeding, the egg-producing ovaries appear as thin-walled gray folded tissue. They are attached to the long, dark kidneys. You might have to push other organs aside to see the ovaries.

The coiled white tube on each side of the kidneys is the oviduct. This is a passage leading the eggs from the ovaries to the cloaca. The cloaca, in addition to passing waste, passes eggs (and sperm in males) out of the body during mating.

In a male frog, the two yellow, bean-shaped testes are located next to the kidneys. Sperm reaches the cloaca through the Wolffian duct.

10. On the diagrams below, label the ovaries and oviduct, and the testes and Wolffian duct.



Excretory System The kidneys are long, dark organs embedded in the back wall. They filter wastes from the blood. In the female frog, the wastes, in the form of urine, pass from the kidneys through the ureter to the cloaca. From the cloaca, the urine passes into special lobes called the urinary bladder. (Do not confuse the ureter with the coiled oviduct in a female.) In the male frog, the Wolffian duct carries the urine from the kidney to the cloaca. The urinary bladder is a two-lobed pouch attached to the cloaca. Urine is stored in the bladder and eliminated through the cloaca.

11. On the frog diagram on page 173, label the kidneys, ureter, and urinary bladder.

Dissect out the frog's stomach. Cut it open and examine the contents.

12. Is the frog a carnivore (meat eater), or a herbivore (plant eater)?

When you have finished your dissection, wrap the frog in the paper towels and dispose of it as instructed by the teacher. If class time permits, you may wish to save the frog for the follow-up dissection.

ANALYSIS

13. Beside each structure listed below, write the system or systems to which it belongs. (Systems: digestive, circulatory, respiratory, excretory, reproductive.)

arteries _____	oviduct _____
cloaca _____	pancreas _____
esophagus _____	pyloric valve _____
gallbladder _____	skin _____
glottis _____	small intestine _____
heart _____	spleen _____
kidneys _____	stomach _____
large intestine _____	testes _____
liver _____	ureter _____
lungs _____	urinary bladder _____
nares _____	veins _____
ovaries _____	Wolffian duct _____

14. How does the length of the small intestine relate to its function in absorbing nutrients?

15. List two organs that produce substances that aid digestion.

16. In what situation would the location of the frog's external nares be an advantage in breathing?

17. During the cold weather of winter, the frog's body temperature cools and the frog becomes inactive. Where does the frog get food when it cannot catch prey?

18. Why do you think the hind limbs are more muscular than the forelimbs?

FOLLOW-UP

Examine one of the frog's eyes. With forceps, lift the upper and lower eyelids to see how they work. Note the thin, transparent lid, called the nictitating membrane. What do you think is the function of this membrane?

Remove the frog's eye by cutting it out of the eye socket. Slit open the eyeball and squeeze out the lens. Describe the lens.

35 Oat Coleoptile Experiment

PURPOSE

To learn what causes plants to grow and how factors affect plant growth.

MATERIALS

Pencil

INTRODUCTION

Over 100 years ago Charles Darwin became interested in the oat plant, *Avena sativa*. When an oat seed germinates, the first thing that emerges from the ground is a sheath called the coleoptile. Darwin noticed that when the oat coleoptile was exposed to light, it quickly bent toward the light source. He wondered how the plant senses the presence of light, and so he performed the following experiment.

Darwin covered the tips of some oat coleoptiles with opaque glass cylinders and covered the tips of others with clear glass cylinders. Those covered with opaque glass did not bend toward the light. Those covered with clear glass did turn toward the light. Darwin reasoned that the tip of the plant somehow controlled the directional growth of the plant. But how?

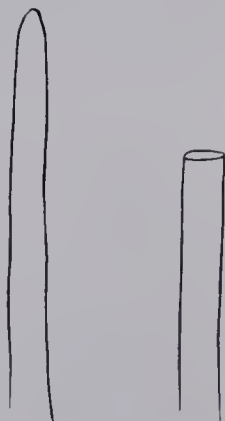
In later years, other experiments were performed to answer this question. Study the experiments that follow, and try to solve the problem.

PROCEDURE

Experiment A

Two groups of oat seeds were germinated in total darkness. In one group, the tips of the coleoptiles were removed. The coleoptiles whose tips were not touched elongated normally. Those without tips did not elongate.

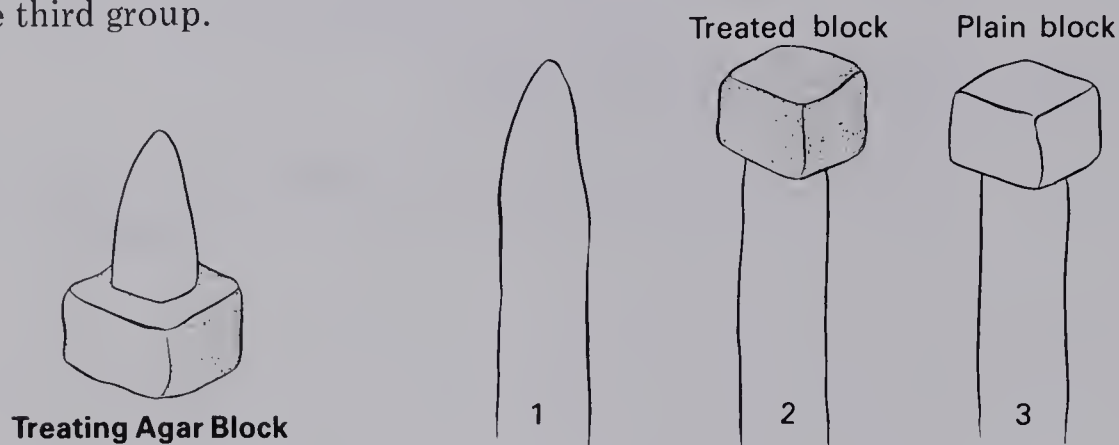
1. How do you interpret the results of this experiment?



Experiment B

First, some agar blocks were treated in the following way. (Agar is made up largely of a polysaccharide, and allows materials to diffuse easily into and out of it.) The tips from some oat coleoptiles were removed. The tips were placed, cut ends down, on small blocks of plain agar. After 24 hours the cut tips were removed.

Next, three groups of oat seedlings were prepared. The first group was not touched. The tips were removed from the coleoptiles in the second and third groups. The treated agar blocks were placed on the tops of the second group. Plain agar blocks were placed on the tops of the third group.

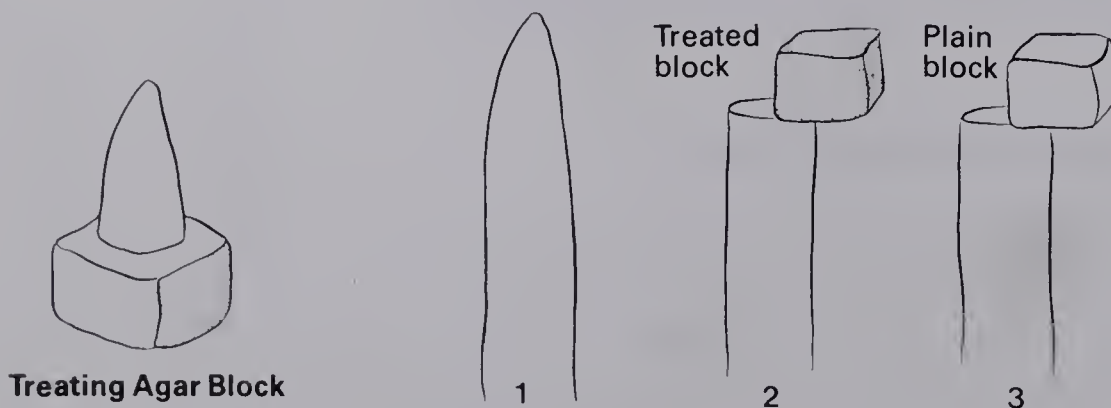


All the plants were kept in the dark. The first and second groups showed normal elongation. The third group did not elongate.

2. How do you interpret the results of this experiment?

Experiment C

Agar blocks were treated as in experiment B. Three groups of oat seedlings were prepared as follows. The first group was not touched. The tips were removed from the coleoptiles in the second and third groups. In the second group, a treated block of agar was placed over half of each cut end. In the third group, a plain agar block was placed over half of each cut end. All plants were kept in the dark.



The plants in the first group elongated normally. The plants in the second group curved toward the uncovered side, so that the treated agar block was on the top of the curve. The plants in the third group did not grow.

3. How do you interpret the results of this experiment?



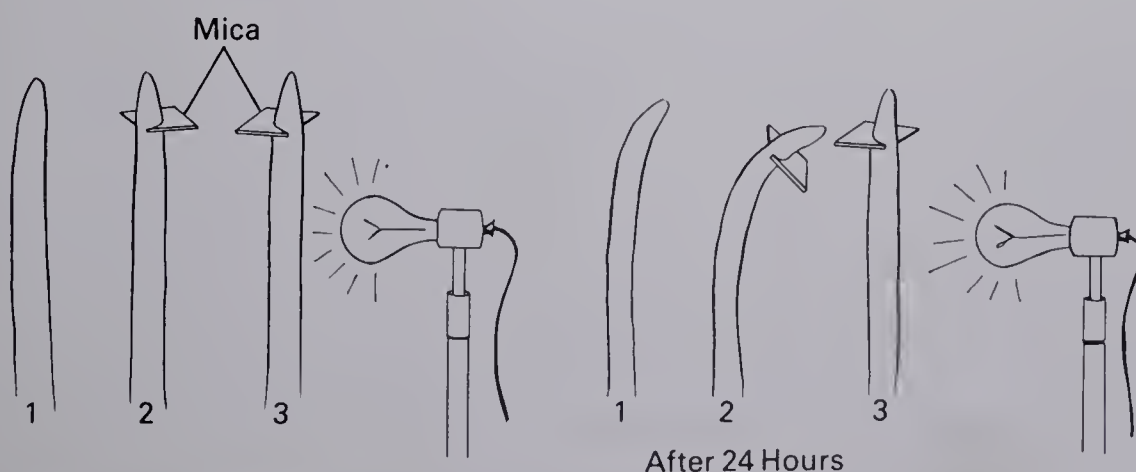
In 1866 a German chemist isolated a compound called indole. One of the related compounds of indole is indole acetic acid. Analysis of agar blocks treated as in experiment B shows the presence of indole acetic acid.

It is now known that indole acetic acid, produced by the growing tips of plants, controls the elongation of cells. So, indole acetic acid is considered a plant hormone. Auxin is the term used for one class of plant hormones. Indole acetic acid, generally referred to as IAA, is one of the most common plant auxins.

Experiment D

Three groups of oat seedlings were prepared as follows. The first group was not touched. In the second group, a thin piece of mica, a mineral, was placed halfway through the coleoptile near the tip of each plant. In the third group, a piece of mica was similarly placed, but on the opposite side of each plant. The plants could not transfer substances through the mica. A directional light was placed so that it shone on one side of the young plants.

After 24 hours in the light, the three groups appeared as illustrated. The untouched group curved toward the light. The second group also curved toward the light. The third group grew straight.



2

5. State the hypothesis that you think was tested in experiment A. What was the control? What was the experimental variable?

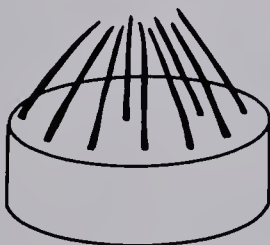
7. State the hypothesis that you think was tested in experiment C. What was the control? What was the experimental variable?

8. State the hypothesis that you think was tested in experiment D. What was the control? What was the experimental variable?

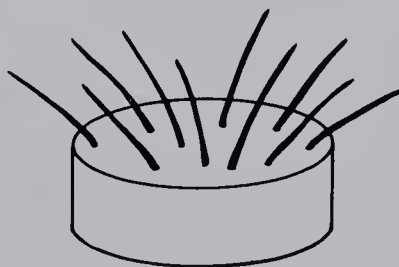
9. A dish of growing oat seeds is placed on a slowly rotating turntable. The light source is ceiling lights. Which drawing—A, B, or C—do you think shows the growth pattern of the seedlings? Why?



A



B



C

FOLLOW-UP

Perform the experiment described in question 9. Plant oat seeds in moist vermiculite in a dish that will fit on a turntable. Use a light dish that will not weigh down the turntable. The seeds may take several days to germinate. When they sprout, place the dish on a turntable rotating at $33\frac{1}{3}$ rpm. Switch on the ceiling lights. The seedlings should show a definite growth pattern in about 24 hours. Do the results confirm what you learned about plant auxins in this lab?

36 Reflex Action

PURPOSE

To demonstrate reflexes and relate them to nervous system structure.

MATERIALS

Part A, per team of 2-4:

flashlight

percussion hammer

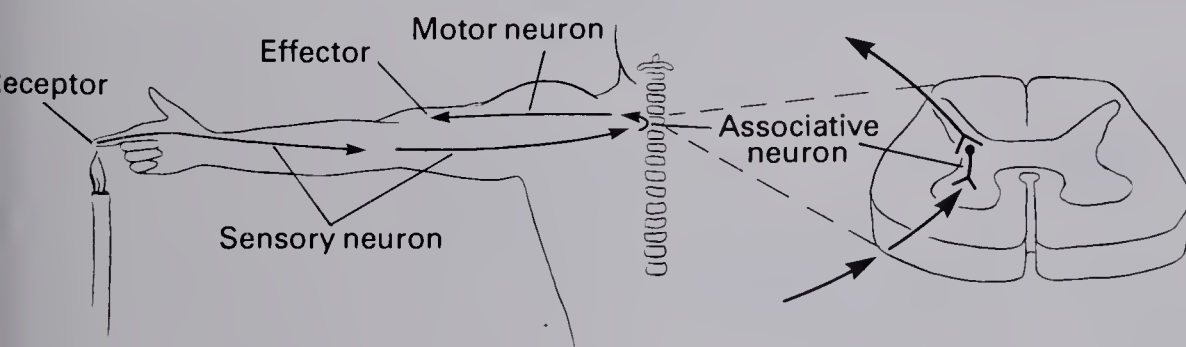
Part B, per class:

dog

INTRODUCTION

When you accidentally touch a hot object, you promptly pull away your hand without thinking. This is a reflex action, an unplanned response to a stimulus.

The heat stimulates receptors in the skin, triggering a nerve impulse. The impulse is carried by a sensory neuron to the spinal cord. There, the impulse passes through an associative neuron to a motor neuron, which carries it to an effector (a muscle or gland). The effector muscle contracts, pulling your hand away from the heat. The whole process is called a reflex arc.



Because the nerve impulse is not transmitted to the brain, no thought is required for a reflex action. However, additional messages do go to the brain, making you aware of the stimulus.

In this lab you will demonstrate reflex actions in a human and a dog.

PROCEDURE

A. Human Reflexes

There are many human reflexes. Drawing the thumbnail along the sole of the foot produces a toe-curling reflex. Touching the inside of the

nostril with a hair should produce a sneezing reflex. Holding a piece of food on the tongue produces a salivation reflex. Waving a hand close to the eyes produces a blinking reflex.

Two reflexes are easily and safely demonstrated in a lab: the pupillary reflex and the patellar reflex. You will work with a partner to test these reflexes, taking turns being the tester and subject.

Pupillary Reflex Human beings are primarily diurnal (active in the daytime), but are adapted to see well in either bright or dim light.

The teacher will dim the room light. Close your eyes and cover them with your hands for at least 30 seconds, then open and uncover your eyes. Your partner should then note how large your pupils are. Next, your partner shines a flashlight in your left eye and watches what happens to the left pupil.

Switch roles and repeat the procedure.

1. How do the pupils respond to light?

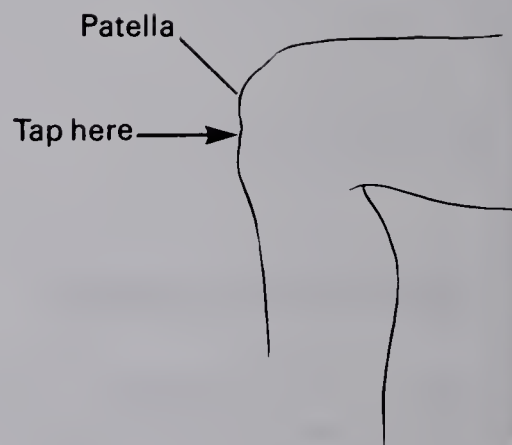
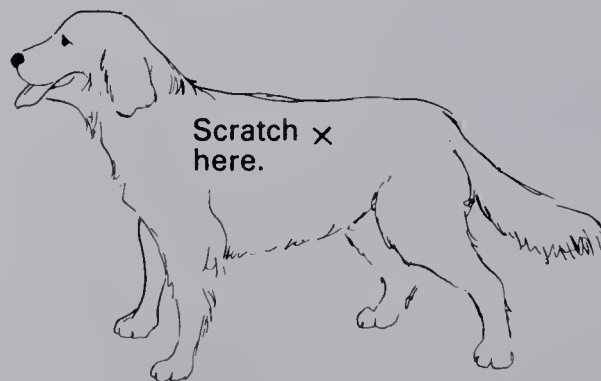
Patellar Reflex Sit on the edge of a table with your feet off the floor. Cross your legs at the knee. Your partner stands to one side and gently taps just below your kneecap with a percussion hammer. If there is no response, tap again in a slightly different place.

Switch roles and repeat the procedure.

2. What is the response?

B. Reflexes in Dogs

All animals with a complex nervous system have reflexes. You will test one reflex in a dog.

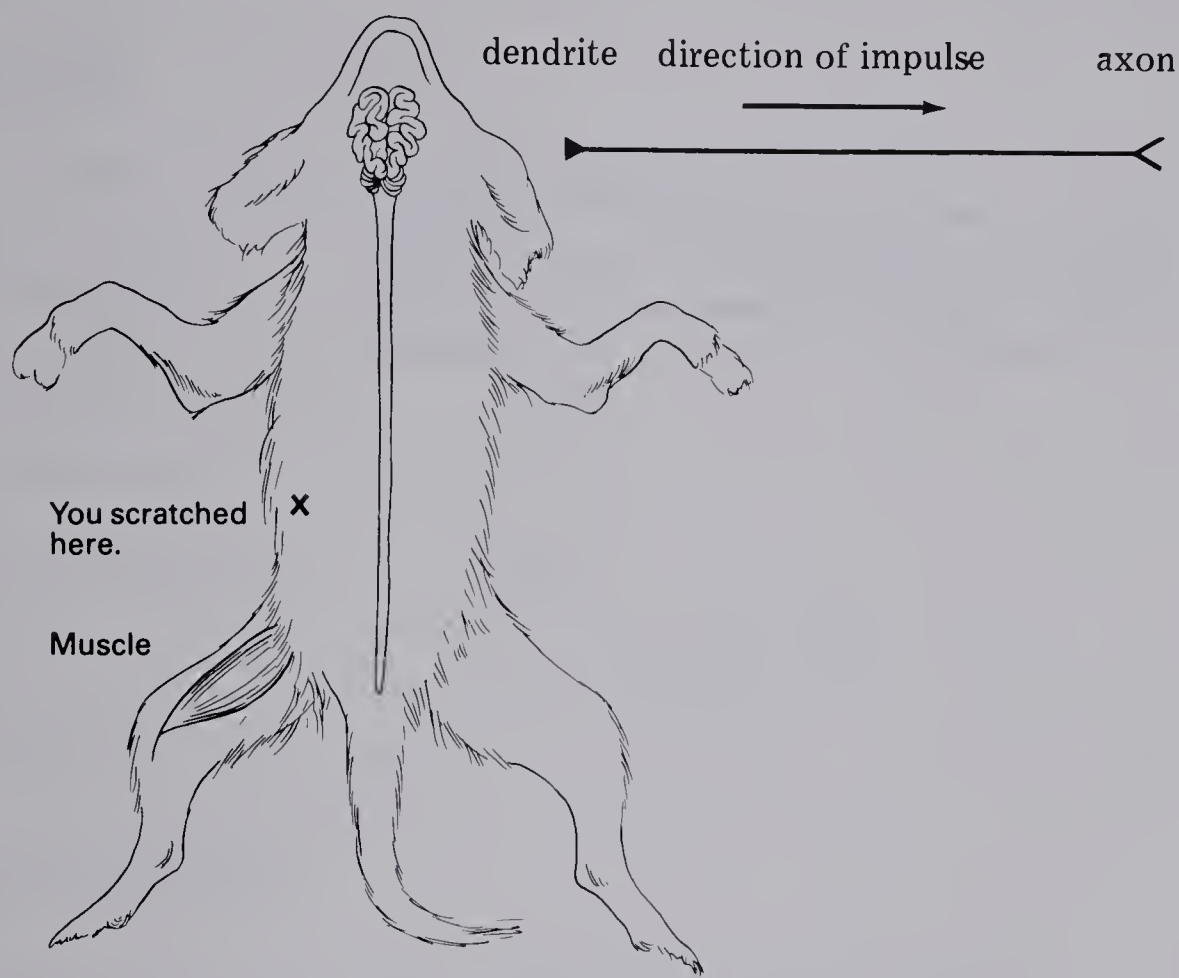


Have a dog lie on its side or stand on a table. Gently scratch the dog's upper side at the point marked in the illustration.

3. What happens?

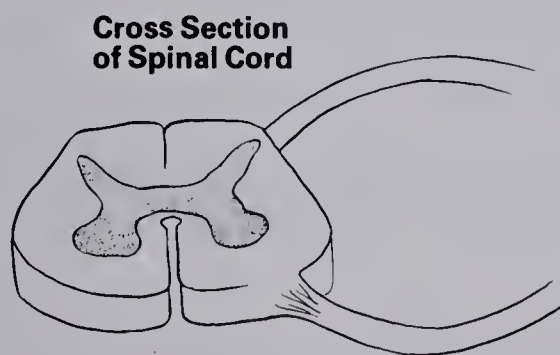
4. On which side is the response?

5. On the drawing of the dog, diagram the reflex arc that produces this reflex. Use these symbols:



ANALYSIS

6. On the drawing below, diagram a typical reflex arc with the symbols you used in question 5.



7. If you had to think to produce a reflex action, how would this affect its speed?

8. When your cornea is touched, you blink. (This blinking reflex is controlled by the brain stem.) How is this reflex an adaptation for survival?

9. Some wearers of contact lenses find that eventually they can “override” the blinking reflex. Describe the pathway in the nervous system that this override takes.

10. Much of human behavior is learned rather than reflexive. People learn habits, which become ingrained parts of their behavior. How does a habit benefit a person in the same way a reflex does? How are habits more advantageous to a person than reflexes?

37 Crayfish Anatomy

PURPOSE

To become familiar with the anatomy of a crayfish.

MATERIALS

preserved crayfish	forceps
dissecting microscope	hand lens
dissecting pan with wax	scissors
dissecting pins	plastic food bag
dissecting probe	

INTRODUCTION

Crayfish are grouped in the phylum Arthropoda, which also includes such animals as insects and spiders. Arthropods are characterized by having jointed appendages and segmented bodies. In crayfish and other higher arthropods each appendage has a specific function. Crayfish, which are aquatic, use their appendages for swimming, walking, food-getting, reproduction, biting, touching, and tasting.

Another striking feature of crayfish is the armorlike shell, called the exoskeleton, that covers the body. This is characteristic of all arthropods. Crayfish belong to the class Crustacea. Other familiar crustaceans are lobsters, crabs, and shrimp.

In this lab you will examine the characteristic external structures of a crayfish, as well as the internal anatomy.

PROCEDURE

A. External Anatomy

Line a dissecting pan with wet paper towels and place the crayfish in the pan with its dorsal side up. Feel the hard exoskeleton. It is made of a substance called chitin.

Body Segments The crayfish's body is divided into two major regions: the abdomen and the cephalothorax, which includes the head and thorax.

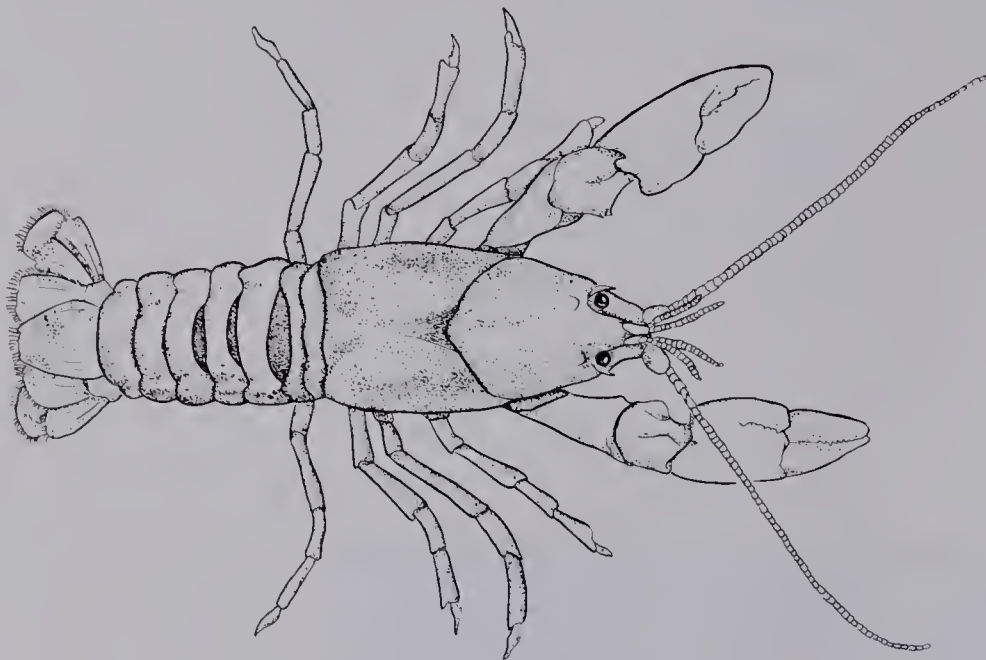
The cephalothorax is covered by a piece of exoskeleton called the carapace. Note the curved cervical groove that marks the division

between the head and thorax. The pointed anterior end of the carapace is the rostrum. Beneath it are the stalked compound eyes.

The segmented abdomen ends in a segment called the telson. Unlike the cephalothorax, the abdomen can be flexed.

1. How might the flexing of the abdomen be useful?

-
2. On the diagram of the dorsal surface, label the cephalothorax, abdomen, carapace, cervical groove, rostrum, eyes, and telson.



Appendages Turn the crayfish over to expose its ventral side. Note the many paired appendages. Crayfish have the ability to regenerate lost body parts, so you may find an appendage that has only partially regrown.

Protruding from the head are two long antennae. Two shorter branched antennules are located between the antennae. The crayfish uses these structures for taste, touch, and smell.

Locate the mouth opening. Surrounding the mouth are jagged jaws called mandibles, used for biting and chewing. Posterior to the mandibles are two pairs of maxillae and three pairs of leglike maxillipeds; these structures are used to hold food. Use a hand lens or dissecting microscope to examine these mouth parts. If you have trouble identifying them, refer to the diagram.

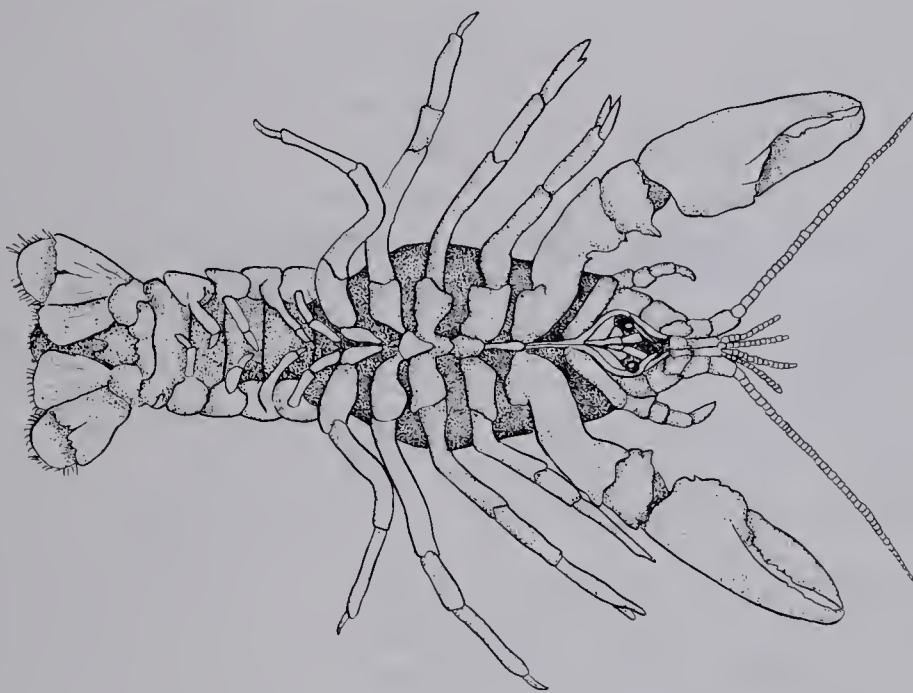
The four pairs of appendages on the thorax are the walking legs. The large, pincerlike appendages are the chelipeds ("pinching legs"), which the crayfish uses for defense and capturing prey.

On the abdomen, note the small appendages called swimmerets. These are used in swimming and reproduction. In a female crayfish the first pair of swimmerets are small. In a male the first two pairs, which transfer sperm to the female, are larger and folded forward.

3. What sex is your crayfish?

At the posterior end, on each side of the telson, are modified swimmerets called uropods. The uropods and telson form a tail fin that is used to propel the crayfish backward through the water.

4. On the diagram of the ventral surface, label the antennae, antennules, chelipeds, walking legs, swimmerets, and uropods.



B. Internal Anatomy

Turn the crayfish so that its dorsal side is up. Insert the point of your scissors just under the carapace at its posterior end. Cut forward along the midline to the rostrum, then cut across the carapace on both sides just posterior to the eyes. Remove the two pieces of carapace.

Take Care: Make shallow cuts, so as not to damage the underlying organs.

Respiratory System Note the exposed feathery gills. Move one of the walking legs and notice how this affects the gills. Pull off the leg and the attached gill.

5. How does the leg attachment aid in the respiratory function of the gill?

Cut out the remaining gills and the thoracic legs. Move one of the maxillae.

6. What is their function?

7. How do the maxillae help in respiration?

Circulatory System Separate the dorsal muscle layer in the thorax to expose the heart, a vase-shaped light-colored organ. The depressions on the heart, called ostia, allow blood to enter. Extending forward and backward from the heart is the dorsal artery.

Remove the heart and the muscles at the sides of the thorax to reveal the organs underneath.

Reproductive System If the crayfish is male, you will see a small pair of white Y-shaped testes below the heart. Sperm ducts extend from the Y to the base of the last legs. If the crayfish is a female, the orange ovaries are likely filled with eggs. Oviducts extend to the second legs.

Mating takes place in autumn. Sperm pass from a male's testes through the ducts to the outside. Using the modified swimmerets, the male transfers his sperm to the female's seminal receptacle, where the sperm are stored over the winter. The eggs are not fertilized until the female lays them in April.

Carefully remove the mass of dark-colored eggs. Insert the point of the scissors under the dorsal side of the abdominal shell and cut back to the telson. Spread the shell to expose the organs underneath.

Digestive System Food enters the digestive tract through the mouth and travels through the short esophagus to the stomach. The large, thin-walled, white stomach is located in the middle of the cephalothorax.

On each side of the stomach are the yellowish digestive glands. They secrete enzymes into the stomach and store food.

Food passes from the stomach into the intestine. This tube lies on top of the abdominal muscles and runs to the ventral surface of the telson. Waste passes out of the body through the anus.

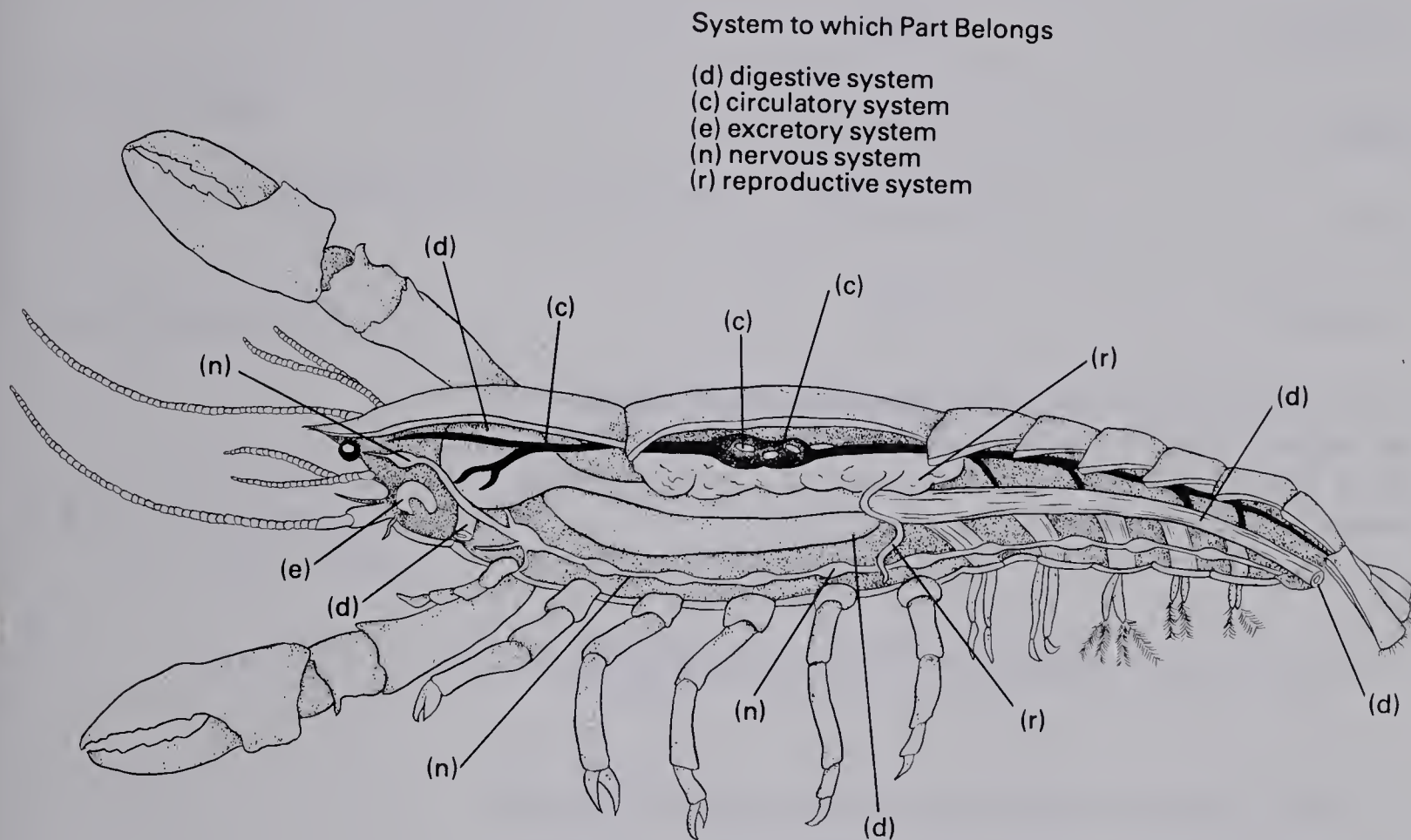
Remove the digestive organs as follows. Cut through the esophagus and the bands of muscle behind the eyes that hold the stomach. Lift out the stomach and any structures that are connected to it.

Excretory System Carefully remove any remaining tissue in the head to expose the large pair of green glands, which are just behind the antennules. The green glands empty wastes into the surrounding transparent bladder. A duct from the bladder opens to the exterior.

Nervous System Carefully cut away the rostrum and any remaining carapace. Between the eyestalks is the brain, a small white mass. Note the nerves traveling from the brain to the eyes and antennae.

Remove any remaining organs in the thorax and abdomen, and cut through the hard plates lining the cephalothorax. Locate the double threadlike nerve cord that extends from the brain along the ventral surface of the body. Where the nerve cord branches are enlargements called ganglia.

8. On the diagram of the internal anatomy, label the heart, ostia, dorsal artery, gonad (ovaries or testes), reproductive duct, esophagus, stomach, digestive gland, intestine, anus, green gland, brain, nerve cord, and ganglia. The system to which each part belongs is noted on the diagram.



ANALYSIS

9. What are the functions of the following appendages?

maxillae _____

maxillipeds _____

chelipeds _____

walking legs _____

swimmerets _____

10. Beside each structure listed below, write the system to which it belongs. (Systems: digestive, circulatory, respiratory, excretory, reproductive, nervous.)

artery _____ heart _____

brain _____ intestine _____

digestive glands _____ nerve cord _____

esophagus _____ ovaries _____

ganglia _____ reproductive duct _____

gills _____ stomach _____

green glands _____ testes _____

11. In the crayfish, blood is pumped into spaces called sinuses around the organs. After delivering oxygen and picking up wastes, the blood drains back into the heart through the ostia. What type of circulatory system is this?

12. To what structure in humans are the digestive glands comparable?

13. To what structure in humans are the green glands comparable?

14. Why is the crayfish grouped in the phylum Arthropoda?

FOLLOW-UP

Each of the crayfish's compound eyes is made up of long visual rods. The outer surface of each rod is called a facet. Light is focused through each facet onto the retina, producing a fuzzy but wide-ranging image. Examine an eye with the dissecting microscope. Note the numerous facets in each eye.

Because the eyes are on movable stalks, the crayfish has a very wide field of view. How might this be an advantage?

The sense of touch is probably more important in the crayfish than vision. Touch receptors are located in specialized hairs on the body as well as in some appendages.

38 Perception of Touch, Temperature, Smell, and Taste

PURPOSE

To learn about perception of touch, temperature, smell, and taste in humans.

MATERIALS

Touch:

compass with two points,
or with one point and
toothpick or sharp pencil

ruler

Taste:

lemon juice

quinine water

5% fructose solution

5% sucrose solution

10% sodium acetate solution

cotton-tipped swabs

Temperature Perception:

container of hot water

container of ice water

container of room-temperature
water

Smell:

small cubes of potato, onion,
and apple

blindfold

paper towels

INTRODUCTION

Information about the environment reaches the brain through sensory neurons. The sensory neurons receive the information from various receptors, which respond to stimuli in the environment. Some of these receptors—those for pain, pressure, position, and touch—are scattered throughout the skin, muscle, or endothelium (lining of the organs). Others—those for taste, smell, sight, and hearing—are concentrated in specialized organs.

In this lab you will investigate the senses of touch, temperature perception, smell, and taste. The parts may be done in any order.

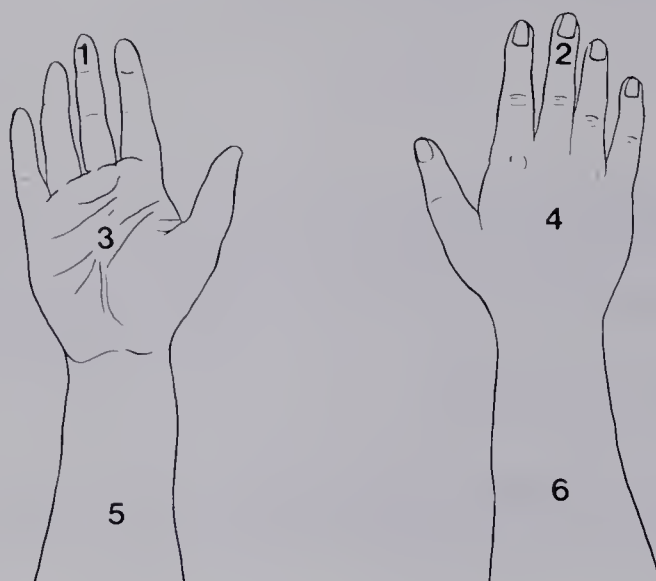
If the workstation for a procedure is crowded, proceed to a less crowded workstation. Be sure to complete all four parts.

PROCEDURE

A. Touch

Humans use their hands in almost every activity. The sense of touch is essential in using the hands and for perceiving stimuli that might be dangerous.

Work with a partner. Set a compass so that the two tips are as close together as possible. Have your partner look away and hold out one hand, palm up. Gently touch the compass tips to your partner's middle fingertip. If your partner feels only one point, widen the compass tips about 2 mm and touch the fingertip again. Repeat until your partner feels two points.



Determine the two-point threshold in the order numbered.

This is called the two-point threshold; it is expressed as the distance between the compass tips. Measure the two-point threshold in millimetres and record the data on the chart in your partner's lab book.

Find your partner's two-point threshold for the back of the middle finger, palm, back of the hand, inside of the forearm, and back of the forearm. Record the data.

Reverse roles and have your partner find and record your two-point thresholds.

<i>Location</i>	<i>Two-point Threshold (in mm)</i>
fingertip (1)	
back of finger (2)	
palm (3)	
back of hand (4)	
inside of forearm (5)	
back of forearm (6)	

B. Temperature Perception

Being able to perceive the temperature of the environment allows humans to avoid painful stimuli and to find environmental areas in which our bodies can function best.

You will test the sensations of hot and cold water. Immerse one hand in the container of ice water and the other in the container of hot water. Keep your hands there for one minute. Then dip both hands at once in the container of room-temperature water.

1. Does the room-temperature water feel hot or cold?

C. Smell

The sense of smell is less sensitive in humans than in other mammals, because the olfactory centers of the brain is much smaller in humans. However, recent research has shown that we unconsciously respond to many odors. In addition, our sense of smell contributes to the flavors of many foods.

Work with a partner. Put on a blindfold and pinch your nostrils shut. Your partner will then put a small cube of potato, onion, or apple in your mouth. Chew the cube and then spit it out into a paper towel. Identify the food. Repeat for the other two foods. Your partner should write down your answers, but not tell you if you correctly identified the foods.

Keep the blindfold on, but do not pinch your nostrils shut. Your partner now gives you the three foods in a different order than in the first test. Breathe as your partner gives you the food so that you will smell the food as well as taste it. Record your answers. Dispose of the towels containing food.

Now switch roles and perform the same two tests.

Nostrils Shut		Nostrils Open	
Food Given	Identification	Food Given	Identification

2. In the first test (with your nostrils closed), could you tell which food was which?

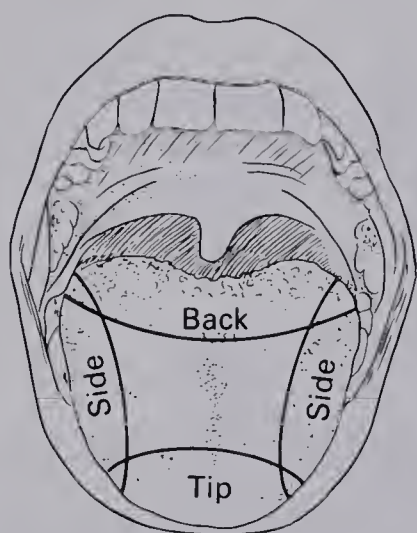
3. In the second test (with your nostrils open), could you tell which food was which?
-

D. Taste

Have you ever been surprised by the taste of orange juice after you brushed your teeth? The reason for the odd taste is that most toothpastes contain detergents like sodium lauryl sulfate. These detergents can interfere with your ability to taste sugar. Orange juice without the sugar taste seems sour and bitter.

There are four categories of taste perception: sour, salty, sweet, and bitter. The chemoreceptors for taste, called taste buds, are located in four areas on the tongue.

The tip of the tongue has a high concentration of taste buds sensitive to *two* kinds of taste. The side regions are most sensitive to *one* kind of taste. The back of the tongue is particularly sensitive to the fourth taste category.



Sour Taste Sour taste results from the detection of hydrogen ions. Acids have a high concentration of hydrogen ions; therefore, acids taste sour. For example, lemon juice is acidic.

Dip a clean cotton swab into the lemon juice. Touch the four regions of your tongue, one at a time.

4. In which area did you perceive the sour taste most?
-

Salty Taste Salty taste results from the detection of positive sodium ions. Table salt is sodium chloride.

Rinse your mouth with water. Dip a clean cotton swab into the sodium chloride solution and touch the four areas of your tongue.

5. In which area did you perceive the salty taste most?
-

Certain negative ions interfere with taste bud reception of positive sodium ions and reduce the taste of saltiness. For example, mono-

sodium glutamate (MSG) contains many sodium ions but does not taste very salty. The negative ions in MSG interfere with sodium ion detection by the taste buds.

Rinse your mouth with water. Test the sodium acetate solution on the salt-tasting area of your tongue. The sodium acetate solution contains just as much sodium as does the sodium chloride solution.

6. Does the sodium acetate taste more salty or less salty than the sodium chloride?

7. Which inhibits the sodium ion detection more—the negative chloride ions or the negative acetate ions?

Sweet Taste Sweet taste results from the detection of molecules that are larger than the ions detected in sour and salty tastes. Several different kinds of molecules produce the perception of sweet taste. Also, the ability to taste different sugars and other sweet-tasting molecules varies from person to person.

Rinse your mouth with water. Dip a clean cotton swab into the sucrose solution and touch the four areas of your tongue.

8. In which area did you perceive the sweet taste the most?

Rinse your mouth, then test the fructose solution.

9. How does the sweetness of fructose compare to the sweetness of sucrose?

10. Compare your answers with those of other students. How many students in your class thought the sucrose was sweeter? How many thought the fructose was?

Bitter Taste As in tasting sugars, tasting bitterness results from the detection of several different kinds of large molecules. Some people are sensitive to certain bitter-tasting molecules, while others can barely detect them. Certain large molecules that stimulate the sugar taste buds also can stimulate the bitter taste buds. This is why some people find artificially sweetened foods too bitter.

Rinse your mouth with water. Dip a clean cotton swab into the quinine water. Touch the four areas of your tongue.

11. In which area did you perceive the bitter taste the most?

12. How is a well-developed sense of taste an adaptation for survival?

13. With your nostrils closed, could you tell which cube was apple, onion, or potato? If you could not, why not?

14. What does your reaction to the room-temperature water tell you about temperature sensation?

15. When you eat vanilla ice cream, what senses are involved?

16. What do your two-point thresholds tell you about the abundance of your touch receptors?

17. Explain the relationship between stimulus, receptor, and perception. Use one of your tests as an example.

FOLLOW-UP

There is a common saying that a blind person's nonvisual senses become better developed in compensation for the lack of sight. However, it seems more likely that the person just relies more on the other senses.

With the knowledge you have gained in this lab, you may be able to suggest ways of using the other senses for tasks that ordinarily require vision. For example, Braille labels are sometimes added to the buttons in elevators.

Suppose you wanted to design a kitchen for a blind person. What design features would help the person to:

- measure foods accurately?

- use recipes?

- use the controls on a stove and refrigerator?

- identify spices and extracts?

- put out a fire?

- take telephone messages?

39 Visual Perception

PURPOSE

To learn how the sense of vision works.

MATERIALS

cellophane or tissue paper
(red and green)

2 flashlights per team

felt-tip markers (red,
yellow, and green)

rubber bands

ruler

INTRODUCTION

Most of our knowledge of the world comes through the sense of vision. This sense encompasses several kinds of perception.

The eyes contain cells called rods and cones, which allow us to see many variations in light and color. Because our two eyes face forward, we see in three dimensions. This stereoscopic vision enables us to perceive depth and judge distance.

Whatever the eye sees is processed by the brain. Images of specific shapes—especially faces—can be remembered and compared with memories of previous images.

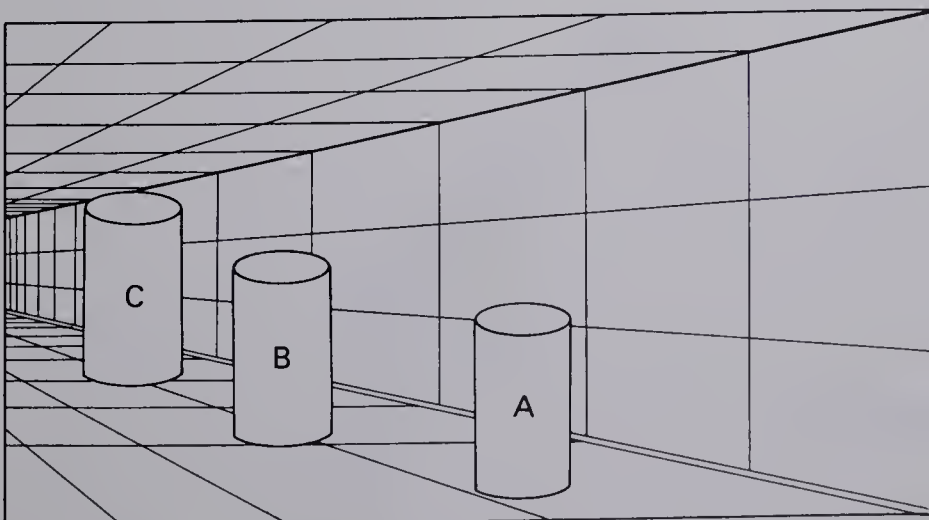
In this lab you will investigate several types of perception that are linked to the sense of vision.

PROCEDURE

A. Seeing in Perspective

Some of the ability to perceive size and distance is inborn. This ability is sharpened by the use of external cues.

Briefly look at the picture of the cylinders.



1. Which cylinder is largest? (Do not measure them.)

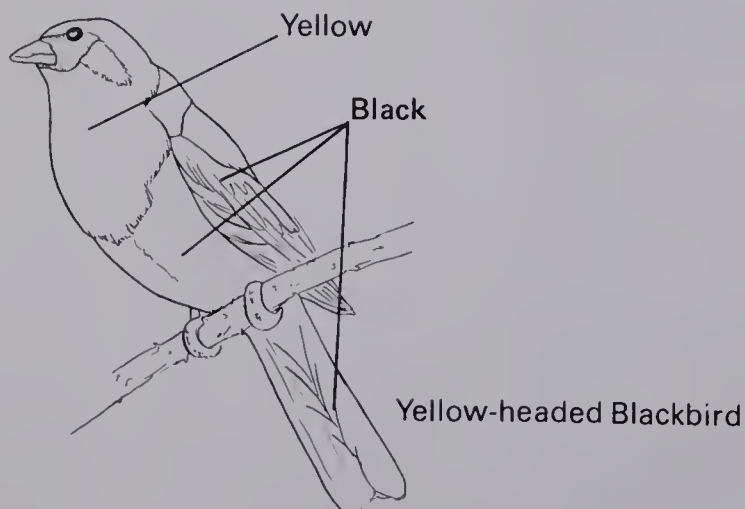
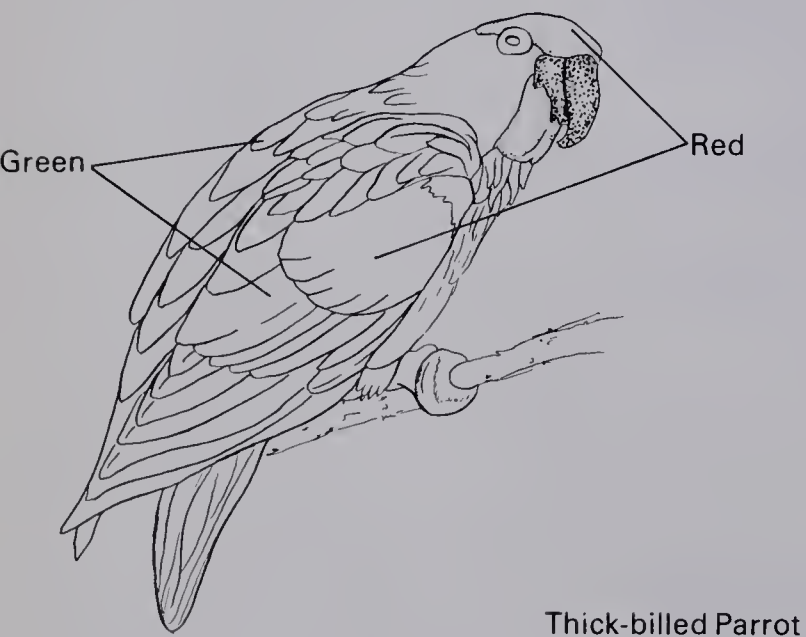
Now measure the cylinders.

2. Were you mistaken? Why might people mistake the sizes of the cylinders?

B. Retaining Images

When you gaze intensely at an image, the image seems to be “burned” onto the retina. After the image is removed from sight, the retina will retain an afterimage.

Use felt-tip markers to color the birds on the left-hand side as labeled. Do not color the birds on the right-hand side.



Stare steadily at the eye of the colored parrot for 30 seconds, then look at the eye of the uncolored parrot. Label the areas of the uncolored parrot with the colors you see.

3. What is the relationship between each color and its afterimage?

This phenomenon is known as retinal fatigue. Apparently, after retinal receptors have received a certain wavelength of light for a long time, they can no longer respond to it. However, the receptors still respond to the other colors of visible light. These combine to produce the complementary color of the color no longer received.

C. Eye Dominance

Just as you are right- or left-handed, you have one eye that is dominant over the other.

Go to a window and point at some small object far in the distance. Holding your hand steady, close one eye, then the other. The eye that remains in line with the object is your dominant eye. You used this eye when you pointed at the object.

4. Which is your dominant eye?

5. Are you right- or left-handed?

Compare your results with those of your classmates.

6. How many people have a dominant hand and eye on the same side? In what percentage of the class is the right side dominant? How do you explain this pattern of dominance?

D. Color Perception

We distinguish most objects from their surroundings more by color than by other characteristics.

Use rubber bands to fasten red cellophane (or three thicknesses of tissue paper) over a flashlight, so that the light appears red. Prepare another flashlight with green cellophane or tissue paper.

Work with a partner. Close your eyes. Your partner holds one light close to your right eye and the other close to your left eye, and turns the lights on. Open your eyes and look straight ahead into the lights.

7. What color do you see?

8. What may account for that result?

Reverse roles and repeat the procedure.

ANALYSIS

9. How is the ability to see in perspective useful?

10. What color afterimage would you see after staring intensely at the following colors?

red _____ green _____

yellow _____ white _____

11. When fighting fish first see each other, they respond with aggressive behavior. But if they are kept apart by clear glass, even though they still see each other, they soon ignore (become habituated to) each other. What visual mechanism might be involved in habituation?

12. Would a person with a dominant left eye most likely kick a ball with the right or left foot? Why?

40 DNA and RNA

PURPOSE

To learn how the components of DNA and RNA molecules interact.

MATERIALS

cellophane tape

scissors

INTRODUCTION

Two nucleic acids play important roles in biology—deoxyribonucleic acid, or DNA, and ribonucleic acid, or RNA.

The building blocks of nucleic acids are nucleotides. Each nucleotide is made up of a sugar, a phosphate group, and a nitrogen base.

The sugar in DNA is called deoxyribose. Its formula is $C_5H_{10}O_4$. The sugar in RNA is ribose. Its formula is $C_5H_{10}O_5$. By comparing the two formulas you should be able to tell what the “deoxy-” part of DNA refers to.

The phosphate groups on all the nucleotides are the same.

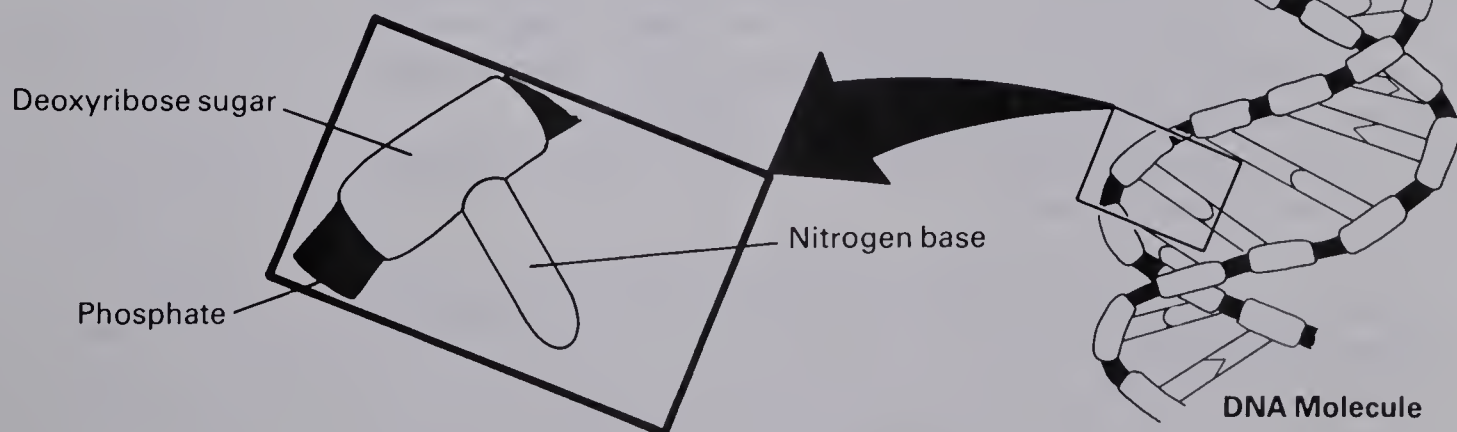
There are four nitrogen bases in DNA and four in RNA. The bases occur in two groups: the purines and the pyrimidines. The purine molecules are larger than the pyrimidines.

In this lab you will construct models to observe the structure of DNA and RNA and how they function in replication and protein synthesis.

PROCEDURE

A. DNA

DNA is made up of two chains of nucleotides that are linked together like a ladder. The sugars and phosphate groups form the sides, and the bases form the rungs.



The four nitrogen bases in DNA are adenine, thymine, guanine, and cytosine. They are usually referred to by their initials: *A*, *T*, *G*, and *C*. Cut out the 24 DNA nucleotides on page 207.

1. Which two bases belong to the purine group?

2. Which two bases belong to the pyrimidine group?

Choose six of the nucleotides, making certain that you have at least one *A*, *T*, *G*, and *C*. Place the six nucleotides face down on your desk and mix them up. Now turn over one nucleotide. This will be the first link of a nucleotide chain (one strand of DNA). *Randomly* turn over the other nucleotides, one by one, and link each to the chain. Tape the chain together along the sugar-phosphate "backbone."

The letter code of your six nucleotides is written in order of their placement on the chain, in groups of three. The code of the illustrated example is: ATT, GCT.

3. Write the letter code of your chain in the space below and on the chalkboard.

When everyone has done this, see if there are any duplications.

4. Probably each chain is unique. Explain why.

You made the chain by linking the nonlettered sides of the molecules—sugars to phosphates. The lettered parts—the nitrogen bases—also fit together. Using the unlinked nucleotides, find out which kinds of bases fit together.

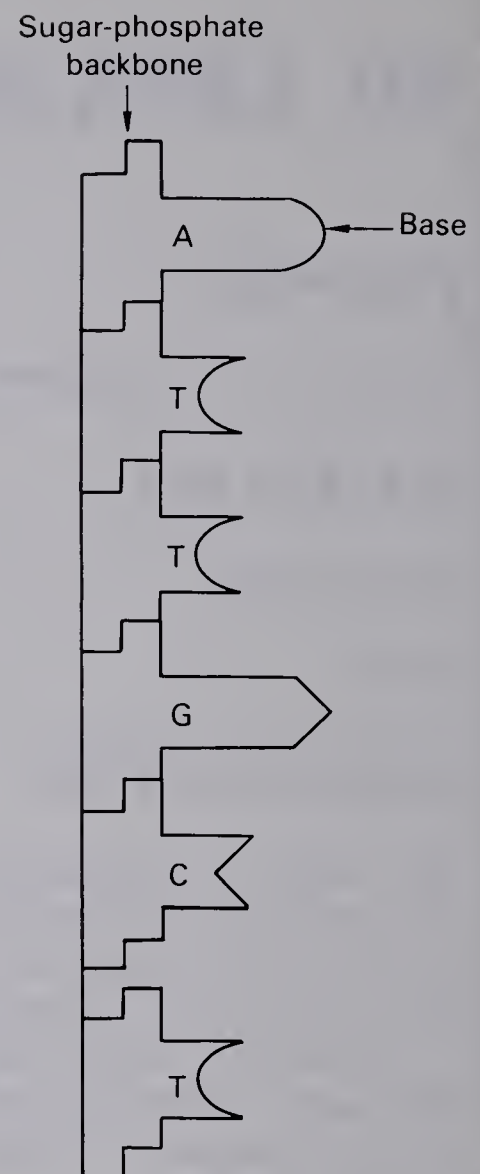
5. What purine-pyrimidine pairs can you form?

Find six unlinked nucleotides whose bases fit the bases of the nucleotides in your six-member chain. As you fit the bases, note that the sugars and phosphates of the new nucleotides link to form another chain. The end result should look like a ladder—a DNA ladder.

Tape the new sugar-phosphate backbone together. Leave the bases (the rung of the ladder) untaped.

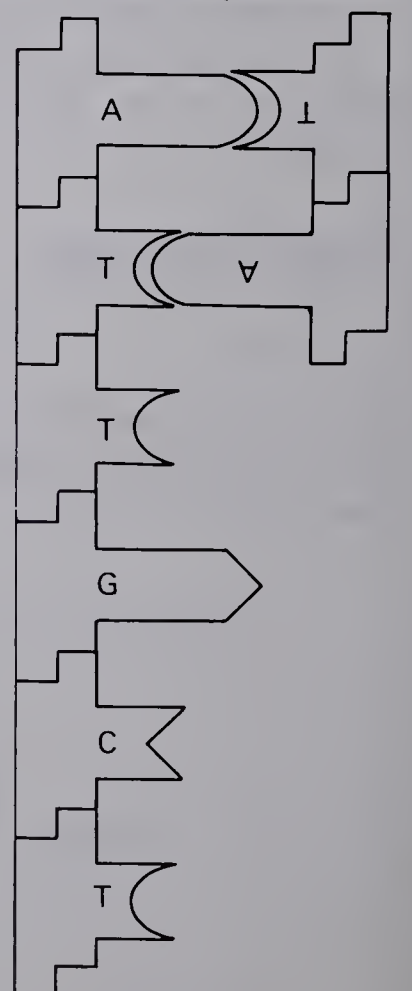
6. Compare the positions of the new nucleotides with the positions of the original ones.

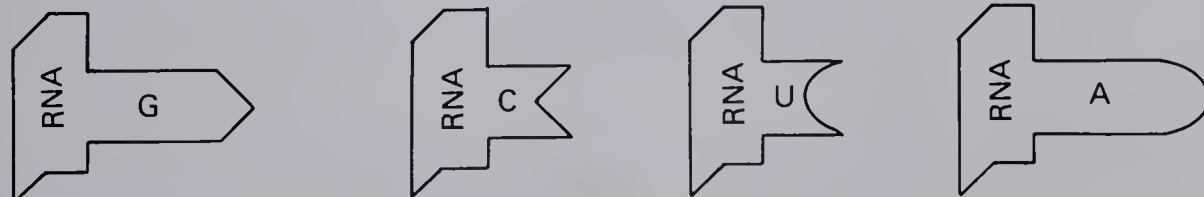
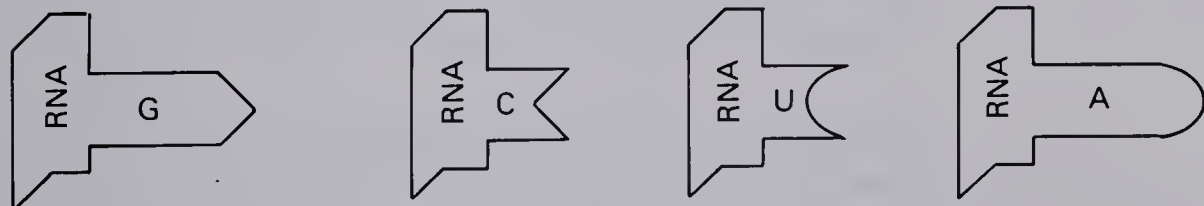
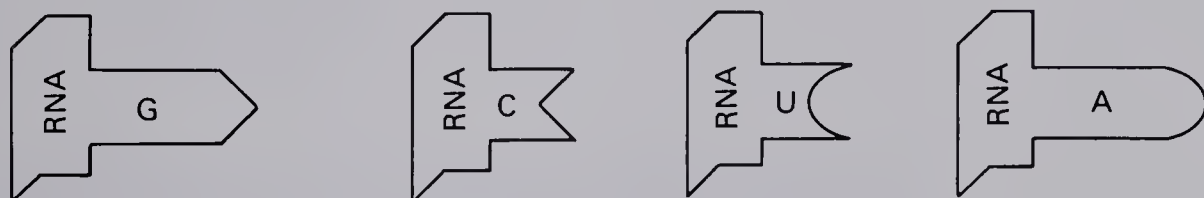
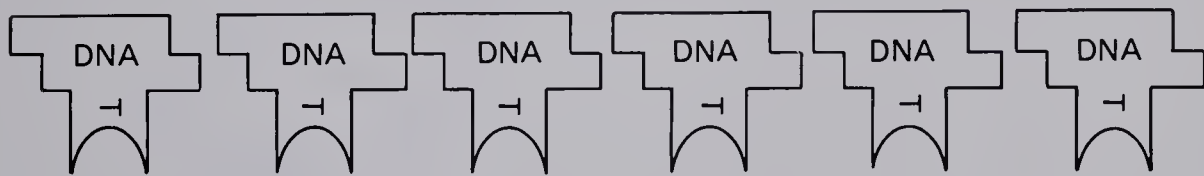
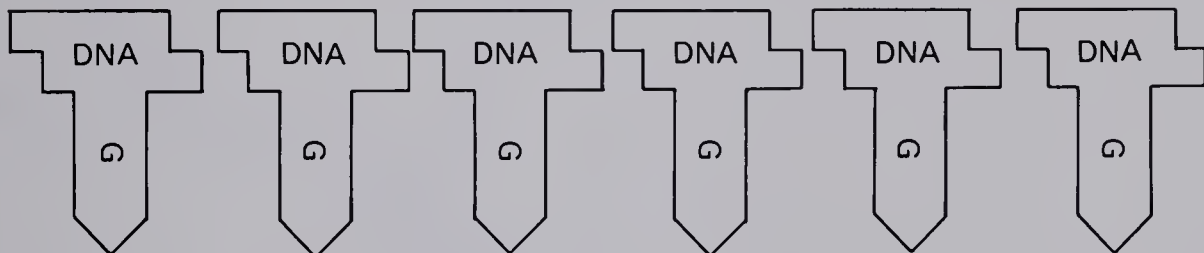
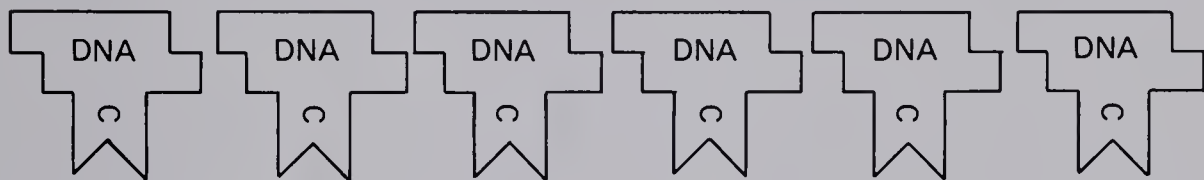
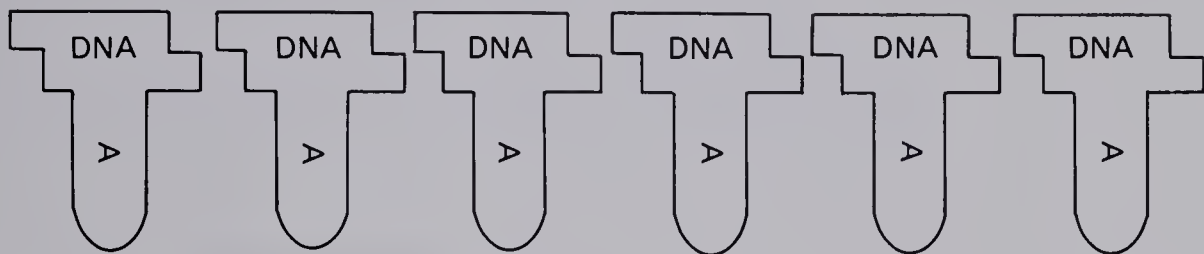
Example of Nucleotide Chain



(The order of nucleotides in your chain will probably be different.)

Fitting Bases

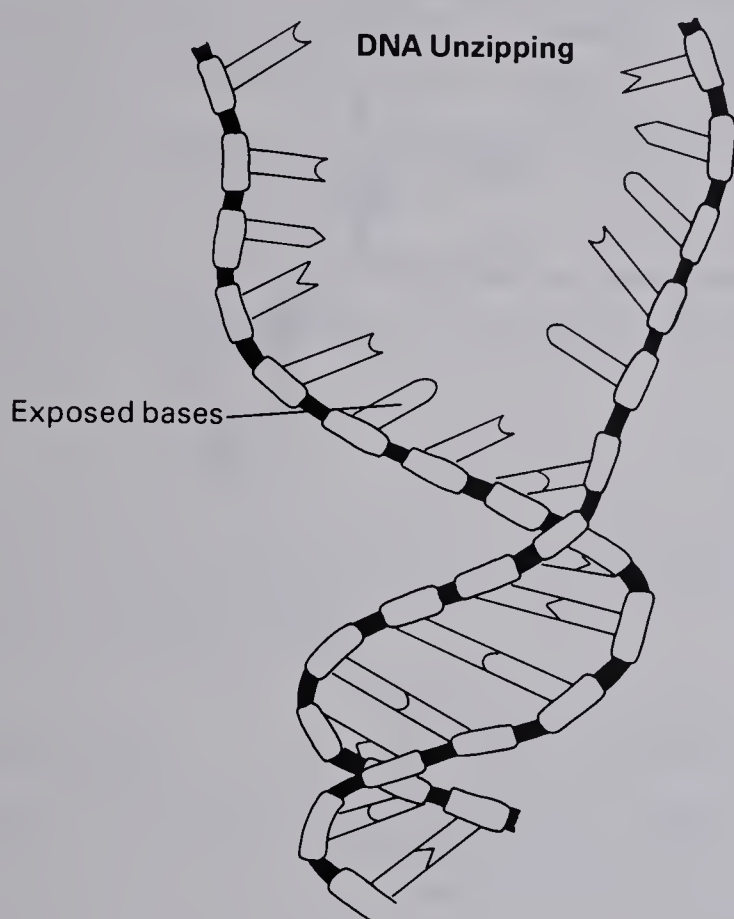




B. DNA Replication

Exactly the same sequences of DNA (packaged in chromosomes) are found in every cell nucleus of an organism. That is possible because before each cell division, each DNA ladder makes a copy—a replica—of itself. The copying process is called replication.

During replication, the DNA molecule “unzips” by breaking the bonds that hold the pairs of bases together. The ladder comes apart at the middle of each rung. As this happens, free nucleotides floating in the surrounding area are attached to the exposed nucleotides of the DNA strand.



You can see how this works on your DNA model. Separate the DNA molecule you made into two halves lengthwise (the untaped part). The nitrogen bases should now be exposed. The sugar-phosphate backbones should remain taped together.

Take the remaining 12 DNA nucleotides that you cut out. Match their bases to the newly exposed bases of your DNA ladder. Continue the process until all the bases are connected. Tape together the sugar-phosphate backbones of the nucleotides you added. Do not tape the bases.

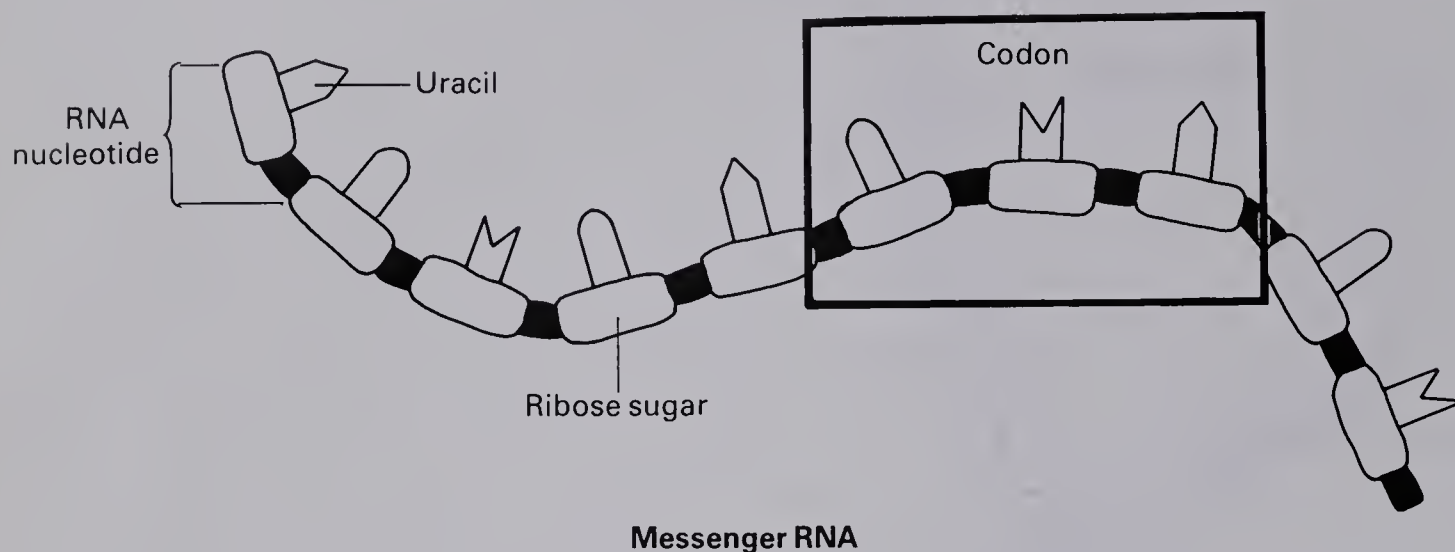
7. How does each new nucleotide chain compare to the one on which it was formed?

8. What is the result of DNA replication?

9. How much of your new DNA chain is from your original DNA molecule?
-

C. Messenger RNA

Messenger RNA (mRNA) carries the DNA code out of the cell nucleus to the ribosomes, and there directs the synthesis of proteins.



The structure of RNA nucleotides differs from DNA. In addition to having a different sugar, RNA has the base uracil instead of thymine.

Messenger RNA is a single strand, formed on one of the halves of the DNA ladder which serves as a pattern. The DNA molecule unzips part way, and the RNA nucleotides attach to one of the DNA strands. Only one strand of DNA is ever "read" by the mRNA.

Cut out the 12 RNA nucleotides on page 207.

10. Which two bases belong to the purine group?
-

11. Which two bases belong to the pyrimidine group?
-

12. Are the purines the same as the DNA purines? If not, how are they different?
-

13. Are the pyrimidines the same as the DNA pyrimidines? If not, how are they different?
-

Unzip one of your DNA ladders. Attach six RNA nucleotides to your one DNA chain, matching the bases. Tape the sugar-phosphate backbone of the RNA strand.

14. Which RNA bases attach to which DNA bases?

Remove the RNA strand from the DNA. Put the DNA chain back together with its matching chain, as before.

Each three-letter nucleotide sequence of the mRNA molecule you just formed is called a *base triplet*, or *codon*. This is the form in which mRNA carries the message, or code, from the DNA to the ribosomes.

15. How many mRNA codons have you formed?

16. List your mRNA codons in the space below and on the chalkboard.

In this lab you made a model of DNA. You replicated your model and used it to form mRNA codons. Your cells do this routinely, as do the cells of every living thing.

ANALYSIS

17. What are the building blocks of nucleic acids?

18. What is replication?

19. How is the formation of RNA similar to DNA replication?

20. How are the two processes different?

21. What is the message that DNA contains and mRNA carries?

FOLLOW-UP

Create a model of protein synthesis. Use the symbols for mRNA provided in this investigation and make your own symbols for the appropriate tRNAs and amino acids. Refer to Table 15-2 on the genetic code on page 449 of the text. Show the entire process of protein synthesis using your model.

41 Cell Division

PURPOSE

To learn about the processes of mitosis and cytoplasmic division.

MATERIALS

prepared slide (longitudinal section) of onion root tip

compound microscope

INTRODUCTION

During cell division, one cell splits and becomes two new cells. The two offspring cells are usually about half the size of the parent cell. During division, the cell does not grow. Growth occurs as the cells use food to build new cell material.

Cell division takes place in two stages. The first stage is mitosis, during which the nucleus divides. The nucleus contains the chromosomes that control the cell's activity.

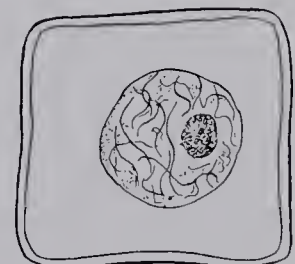
The second stage is cytoplasmic division. The cytoplasm divides, separating the two nuclei and their chromosomes into two new cells.

The entire process of cell division is continuous, and takes about ten minutes in many types of cells.

Mitosis can be divided into four phases: prophase, metaphase, anaphase, and telophase. The phase between cell divisions is called interphase; cells spend most of their time in this phase. You can use the acronym IPMAT to remember the order of the phase. (An acronym is a word made of the initial letters of several words.)

Interphase When a nucleus is not dividing, the chromosomal material is threadlike and webbed throughout the nucleus.

Prophase During prophase, the chromosomes become thick and rod-like. By the time the individual chromosomes are visible, they have already replicated, or made copies of themselves. Thus each chromosome has a double. The doubled chromosomes are held together at a point called the centromere. Meanwhile, spindle fibers, made of protein, form in the cytoplasm.

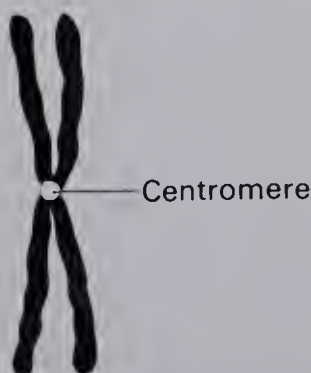


Interphase



Prophase

Replicated Chromosome



Metaphase The doubled chromosomes move to the middle of the cell, to the position called the equator. The spindle fibers attach to the chromosomes at the centromeres.

Anaphase Each chromosome separates from its double, and is pulled by spindle fibers to opposite ends of the cell. As a result, each end of the cell contains an identical, complete set of chromosomes.

Telophase New nuclear membranes form around each set of chromosomes, and the spindle fibers break up.

Cytoplasmic division then occurs, completing telophase and the process of cell division.

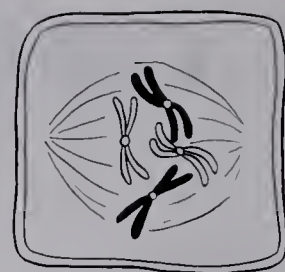
In this lab, you will observe this entire process.

PROCEDURE

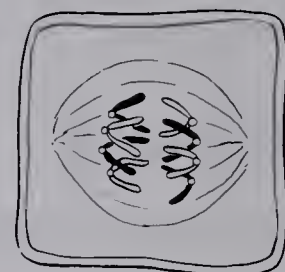
Obtain a slide of an onion root tip. Using low power of the compound microscope, locate the very tip, where you are most likely to see cells that were dividing when the slide was made. Switch to high power.

Look for cells in interphase and the phases of mitosis, and cytoplasmic division. If you cannot find at least three different phases of mitosis, get another slide.

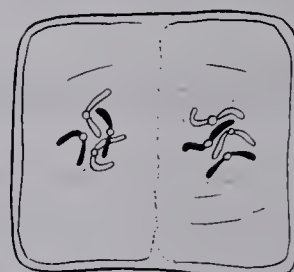
1. Draw and label all the stages you observed.



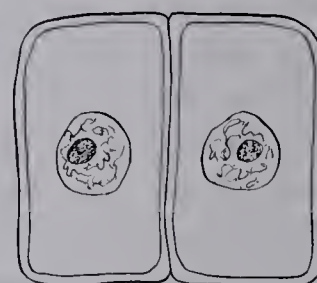
Metaphase



Anaphase



Telophase



Cell Division
Completed

ANALYSIS

2. What are the phases of mitosis called? What is the reason for the name of each phase?

3. How are mitosis and cytoplasmic division different?

4. Mitosis produces two new nuclei. What makes the nuclei exact copies of the original?

5. Do cells grow during cell division? If not, when does growth occur?

FOLLOW-UP

Examine slides of mitosis in animal cells. In what ways are the events like those in plant cells? What differences do you see?

42 Studying Human Chromosomes

PURPOSE

To learn how to prepare and interpret human karyotypes.

MATERIALS

cellophane

scissors

INTRODUCTION

There are several genetic disorders that cause disease or death. However, changes can sometimes be made in the environment to compensate for the genotypes that cause these problems. For example, a child with **phenylketonuria** cannot metabolize the essential amino acid phenylalanine, which is found in milk. Phenylalanine builds up in the body and mental retardation results. But if phenylalanine in the diet is reduced in time, the retardation is not as severe.

To determine whether an individual has a genetic disorder, geneticists first must find out how many and what kind of chromosomes the individual has. Cells used for chromosome studies must be undergoing mitosis. When a cell is not dividing its chromosomes are stretched out in a threadlike form and cannot be identified. During mitosis the chromosomes become short and thick and are easier to identify. Each chromosome has a distinctive shape and banding pattern, and its centromere is at a characteristic place.

Each normal human body cell contains 23 pairs of chromosomes. A pair of chromosomes has matching patterns of dark and light bands, which are composed of genes. Also, each gene on one chromosome of the pair has a corresponding gene (for the same trait) on the other chromosome. Such matched chromosomes are referred to as **homologous**. One comes from the person's father and the other from the mother.

The twenty-third pair, the sex chromosomes, determine sex. A female embryo has two X chromosomes (XX), one from each parent. A male embryo has an unmatched pair (XY), a Y chromosome from his father and an X chromosome from his mother.

The other 22 pairs of chromosomes, called **autosomes**, are all homologous pairs. One member of each pair comes from each parent.

Geneticists are learning the locations of many genes on various chromosomes. They are constructing genetic maps to show those

locations. By examining the banding pattern on the chromosomes (and through chemical tests), geneticists can identify many genetic disorders.

Chromosome number can also be used to identify disorders. For example, during the formation of gametes by meiosis, the chromosomes sometimes do not separate properly. Part or all of a chromosome clings to its homologue. One resulting gamete contains more and the other contains fewer chromosomes than it should have. If either gamete becomes part of a zygote, the resulting embryo will be abnormal. Down's syndrome, which causes mental retardation, results from an extra twenty-first chromosome in the embryo. Turner's syndrome is the result of only one chromosome in the twenty-third pair. If only the X chromosome is present, the person is an abnormal, underdeveloped female.

In this lab you will carry out a procedure called karyotyping (*karyo* means "nucleus"). An individual's karyotype is a chart showing all the individual's chromosomes arranged in order. You will make karyotypes for two people.

PROCEDURE

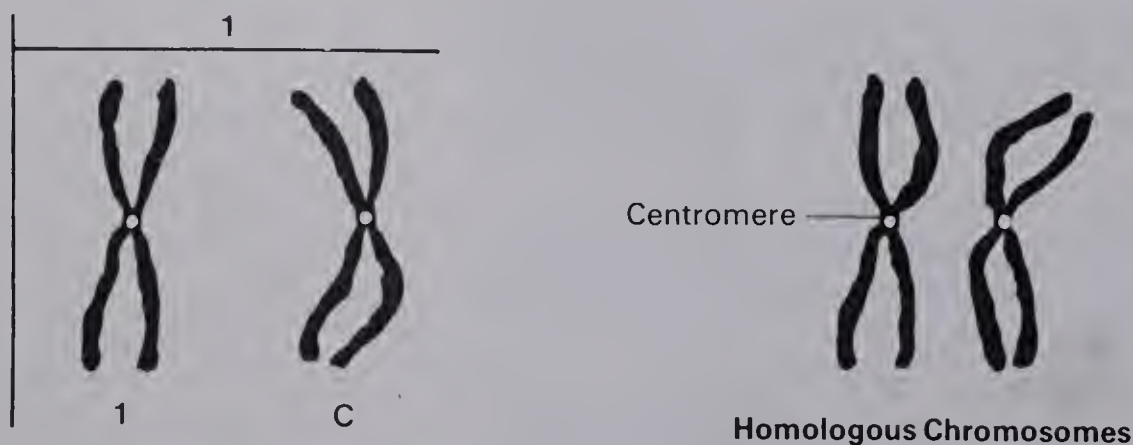
Assume that after examining a person's cells under a microscope you have found a dividing cell and photographed its chromosomes. In addition, you have already examined the chromosomes and given a number to one of each pair. The remaining chromosomes are labelled with letters for identification. The letters X and Y are used for the X and Y chromosomes.

A. Karyotype A

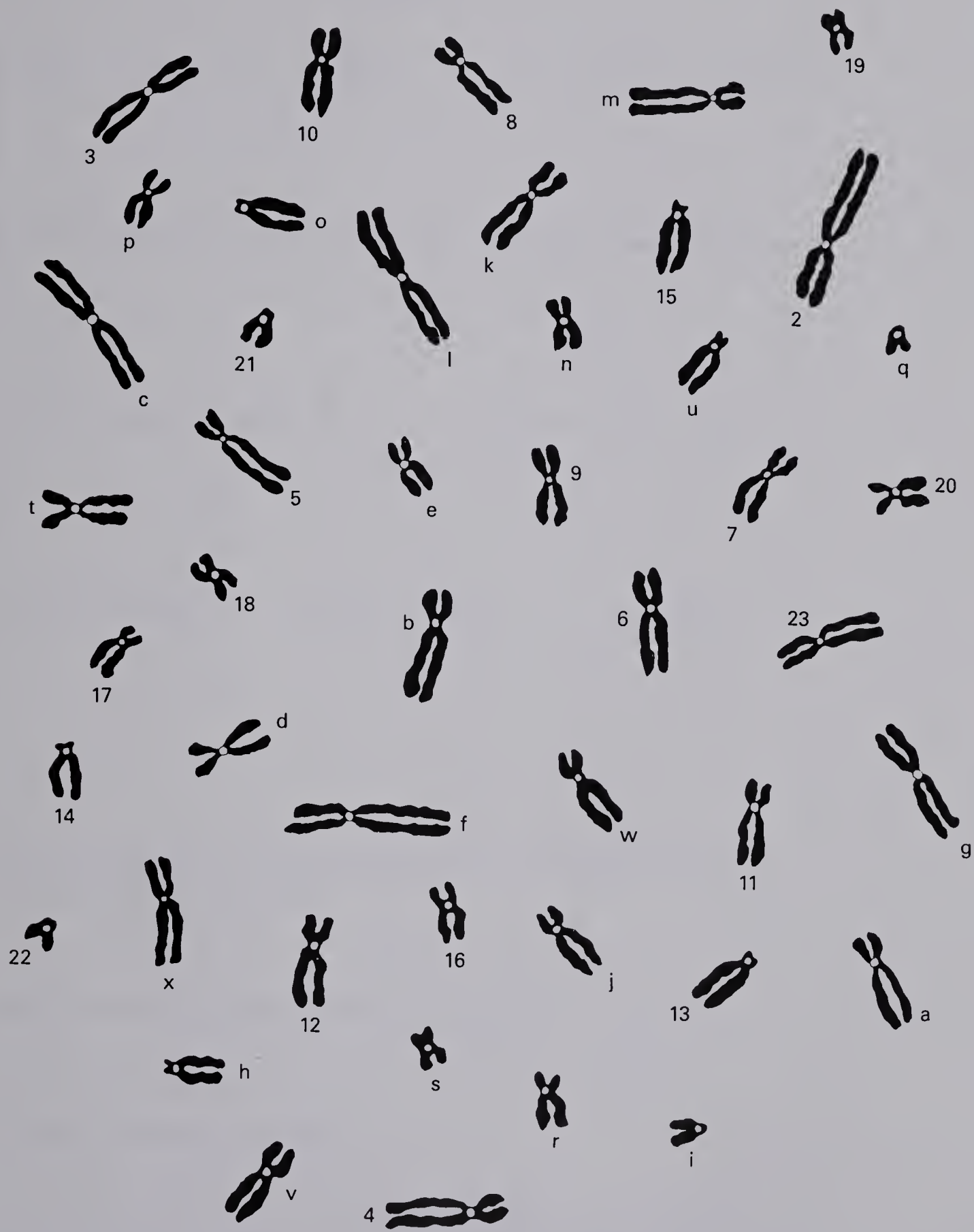
Cut out all the chromosomes for person A, being sure to include the number or letter with the chromosome. Tape each numbered chromosome on the chart for karyotype A on page 221 under the corresponding numbered line.

Next, match the lettered chromosomes to their numbered homologous chromosomes and tape them in place. The location of the centromere, where the two halves (*chromatids*) are linked, should help in identification. The chromatids are the two chromosomes made by replication of DNA prior to mitosis.

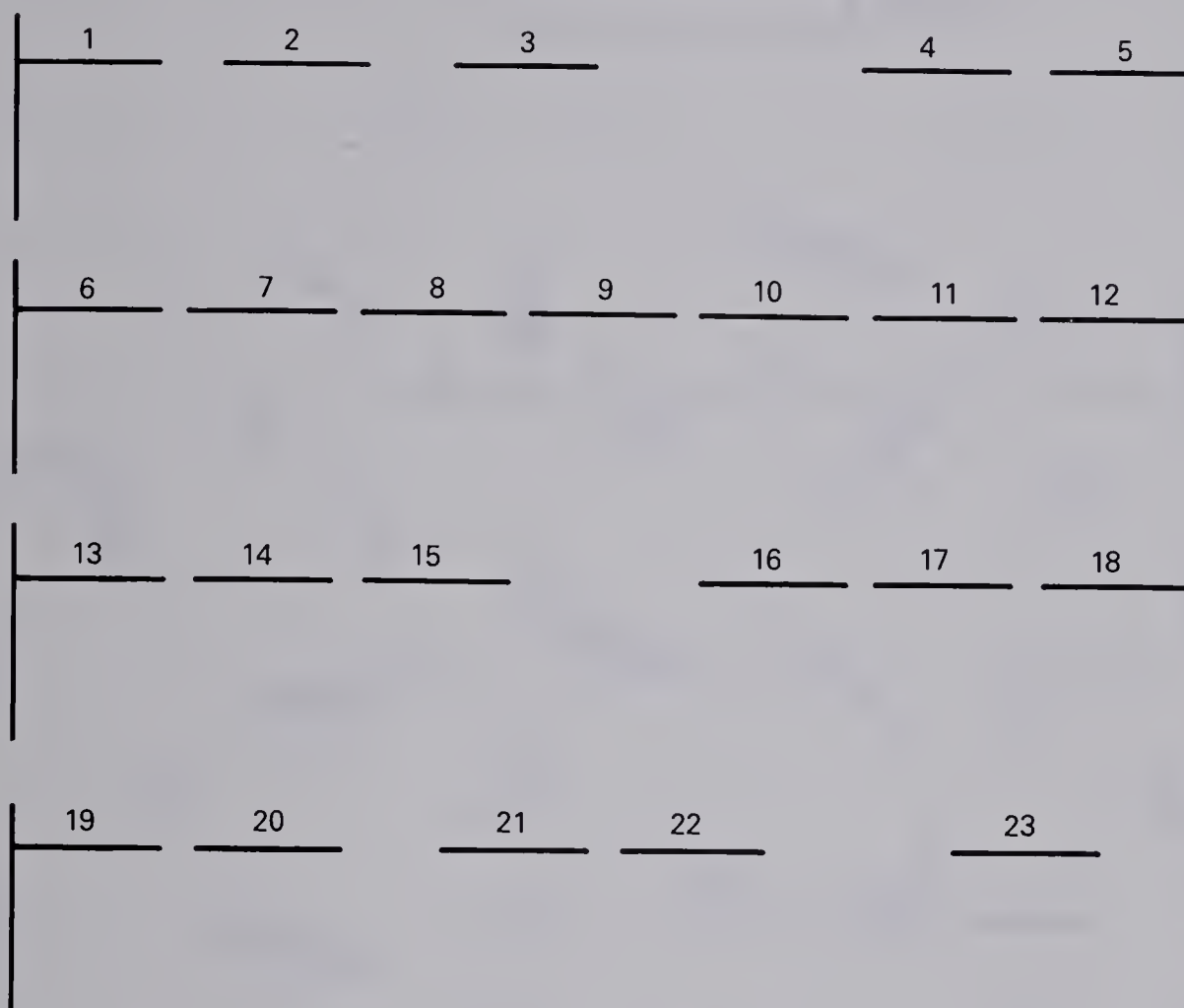
The result should look similar to this example:



Chromosomes of Person A



Karyotype A



1. What characteristics of the chromosomes are the most helpful in matching the homologous pairs?

2. Is the person male or female? How do you know?

3. How many autosomes are present?

4. Can you identify any genetic disorder in this person? If so, what?

When you finish working with karyotype A, put it aside. Be sure that the chromosomes are taped securely, so that they do not become mixed with the chromosomes of karyotype B.

B. Karyotype B

Follow the same procedure you used with karyotype A. Cut out the chromosomes for karyotype B. Tape the chromosomes on the chart for karyotype B.

Karyotype B

1	2	3		4	5	
6	7	8	9	10	11	12
13	14	15		16	17	18
19	20	21	22		23	

5. Is the person male or female? How do you know?

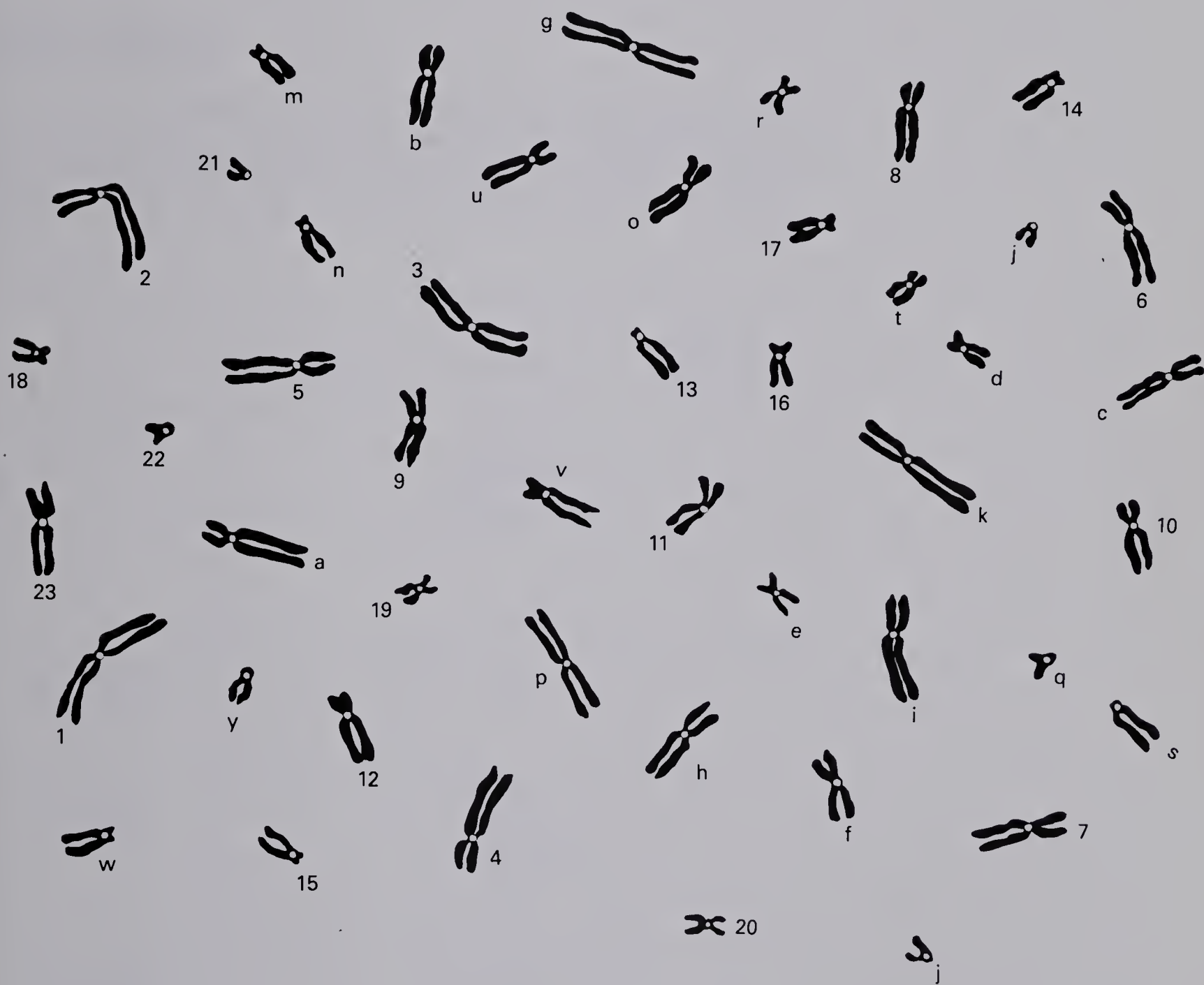
6. How many autosomes are present?

7. Can you identify any genetic disorder in this person? If so, what?

ANALYSIS

8. Suppose that karyotyping revealed that a person is not able to produce enzyme X. The enzyme is needed for metabolizing protein Y. Without the enzyme, the person will develop a serious illness. How could knowledge of the genotype be helpful?

Chromosomes of Person B



9. Different species have different numbers of chromosomes. For example, a fruit fly has 8 chromosomes in each cell and a chicken has 78. Could different species such as these mate and produce offspring? Why or why not?
- _____
- _____

FOLLOW-UP

Some genetic disorders, with their corresponding genotypes, are listed in the chart below. Use reference materials to find the symptoms of each disorder. Decide what the essential clue for diagnosis might be—a feature of the karyotype (including banding pattern), or a chemical test.

<i>Disorder</i>	<i>Symptoms</i>	<i>Genotype</i>	<i>Clue for Diagnosis</i>
Hemophilia		Recessive gene on X chromosome	
Klinefelter's syndrome		XXY	
Tay-Sachs disease		Recessive gene on chromosome 15	
Thalassemia		Loss of a gene on chromosome 11	

43 Mosses and Liverworts

PURPOSE

To become familiar with bryophytes and alternation of generations in plants.

MATERIALS

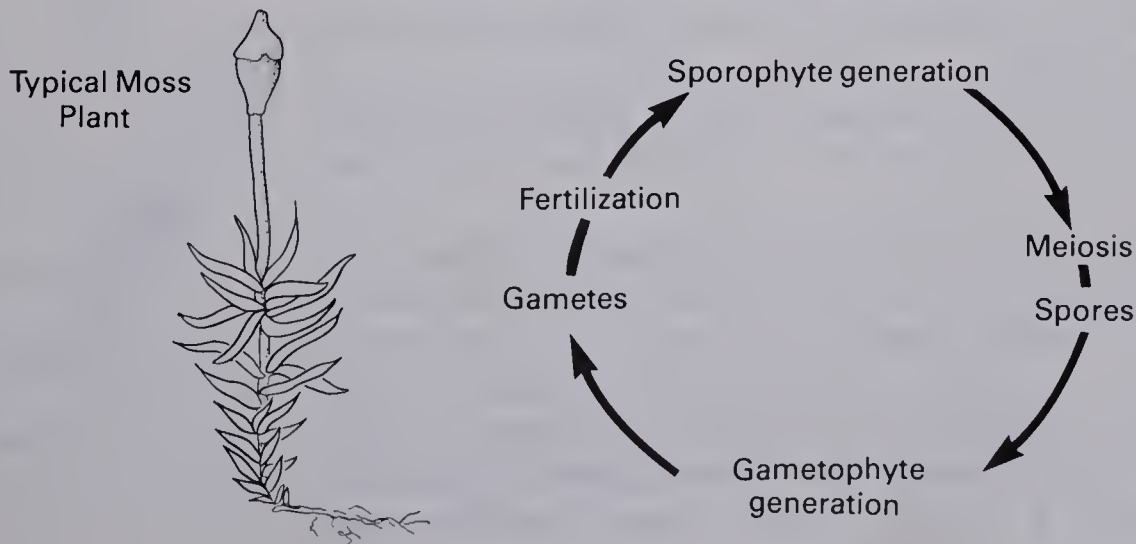
- | | |
|--|-------------------------------------|
| living <i>Marchantia</i> plants | compound microscope |
| living <i>Polytrichum</i> plants | dissecting microscope and hand lens |
| prepared slides of <i>Marchantia</i> archegonia and antheridia | dissecting needle |
| prepared slide of moss protonema | |

INTRODUCTION

Mosses and liverworts belong to a group of plants called bryophytes (*bryo* means “moss”; *phyte* means “plant”). Mosses are small, upright, low-growing plants. Liverworts are even smaller, and grow flat on the ground.

Bryophytes have no vascular tissue or “plumbing” to carry water throughout the plant. To reach the top cells of bryophytes, water and nutrients from the soil must diffuse by osmosis up through all the cells underneath. This method is very slow compared to the swift transport of water through vascular tissue.

Like most land plants, bryophytes have a life cycle with both a sexual stage and an asexual stage. In the sexual stage, the plant produces gametes. Consequently, this stage is called the gametophyte generation. In the asexual stage, the plant reproduces by means of spores, which are produced by reduction division, or meiosis. Consequently, this stage is called the sporophyte generation.



Spores grow into the plants of the gametophyte generation. Gametes unite to produce the plants of the sporophyte generation. This pattern is called alternation of generations.

The gametophyte plants are attached to the ground and carry on photosynthesis. The sporophyte plants are attached to the top of the gametophyte plants and carry on little photosynthesis. Because the sporophyte is dependent on the gametophyte, the gametophyte generation is said to be the dominant generation in bryophytes.

PROCEDURE

A. Mosses

Examine the *Polytrichum* moss plant. The gametophyte generation is made of a dense mat of small, green structures called leaflets. Because the leaflets have no vascular tissue, they are not true leaves. However, they do photosynthesize.

At the base of the gametophyte plant are rootlike structures called rhizoids. They absorb water and nutrients from the ground.

1. How are the water and nutrients transported through the plant?

In many common mosses, the male and female sex organs are located on different plants. The male sex organs are called antheridia, and the female sex organs are called archegonia. Plants that have separate sexes are called dioecious (*di* means “two”; *oecious* means “houses”). Plants with both sexes on the same plant are called *monoecious*. Some mosses are monoecious.

In the process of fertilization, the sperm from the antheridia must swim through water to the eggs in the archegonia. The zygote (fertilized egg) develops in the archegonium.

2. In what kind of environment must a moss live in order for fertilization to take place?

The diploid zygote grows into the diploid sporophyte plant. Sporophytes can be found on the top of some of the gametophyte plants. The sporophyte appears as a stalk topped by a capsule. The capsule contains sporangia, which produce spores. Since meiosis occurs in the sporangia, the spores are haploid.

Pull one sporophyte off the moss plant. With a dissecting needle, remove the capsule's top, the operculum. Use a hand lens to examine the sporangia inside.

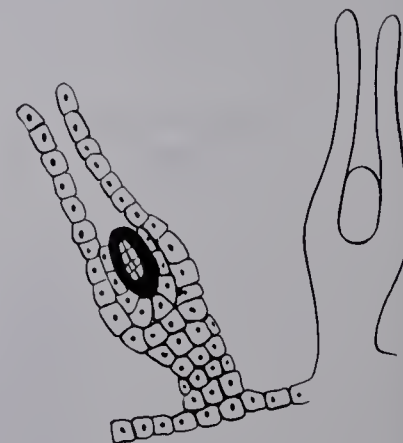
When the sporangia dry out, they open and release the spores into the air. The spores are carried by the wind. Those that land in moist, shady locations will germinate and begin the new gametophyte generation.

When a haploid spore germinates, it produces a filament of cells called the protonema (*proto* means “first”; *nema* means “thread”). The protonema resembles a filament of green algae. Eventually, the

Moss Gametophyte Sex Organs



Antheridia



Archegonia

protonema forms branches that produce rhizoids and buds. The buds open into leaflets, forming the upright structure of the haploid gametophyte plant. Thus, the cycle is complete.

Examine the prepared slide of the moss protonema under the compound microscope.

3. Draw one protonema.

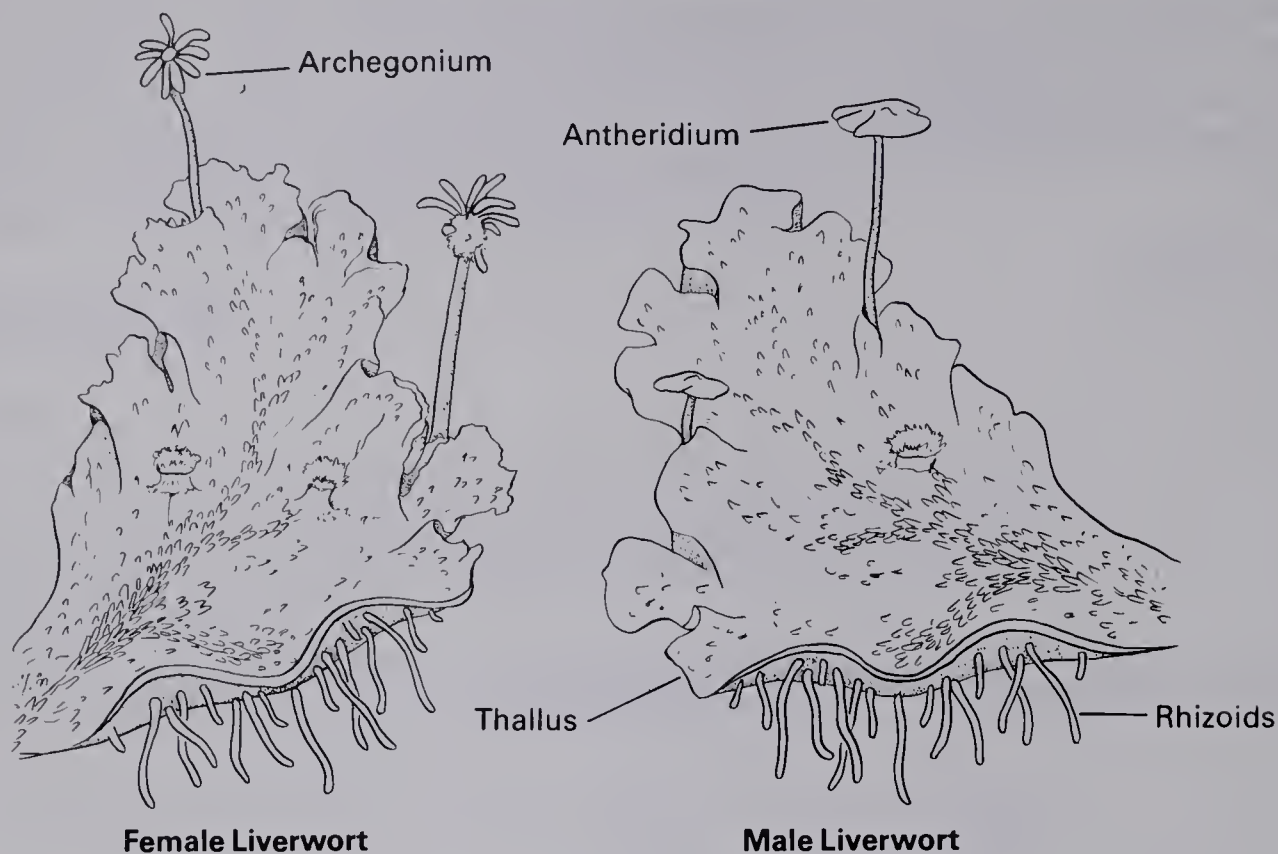
4. On the diagram of the moss life cycle, label each structure.



B. Liverworts

With a hand lens or dissecting microscope, examine the living *Marchantia*, a liverwort. Each leaflike structure is called a thallus. The lobed shape of the thallus resembles a liver; hence the name "liverwort." Note that the thalli are flat instead of upright like moss leaflets. On the underside of each thallus you will see the threadlike rhizoids.

The sex organs of liverworts are elevated on stalks above the thalli. Male and female organs usually occur on different plants.



Observe the prepared slide of *Marchantia* archegonia under a compound microscope. The archegonia will appear as upside-down vases. The widest portion of each vase contains a single egg (usually stained red in prepared slides).

5. Draw one archegonium.

Next, observe the prepared slide of *Marchantia* antheridia. They are oval-shaped structures attached to the plant by a short stalk. Filling the ovals are numerous sperm (usually stained black in prepared slides).

6. Draw one antheridium.

The zygote in the archegonium grows into the sporophyte plant. The sporophyte is attached by a "foot" to the underside of the archegonium. A short stem connects the foot to a capsule, which contains spores.

Examine the living *Marchantia* with a hand lens or dissecting microscope. Look for the sporophytes.

ANALYSIS

7. Describe the process by which bryophytes go from the gametophyte generation to the sporophyte generation.

8. Describe the process by which bryophytes go from the sporophyte generation to the gametophyte generation.

9. Could a moss gametophyte plant survive if its sporophyte plant died? Why or why not?

10. Why is the gametophyte generation considered the dominant generation in bryophytes?

11. Some gametophyte plants never develop sporophyte plants. This may be due to heredity or environmental conditions. In what kind of environmental conditions would a gametophyte plant not develop a sporophyte? Explain your answer.

44 Ferns

PURPOSE

To become familiar with ferns and understand their life cycle.

MATERIALS

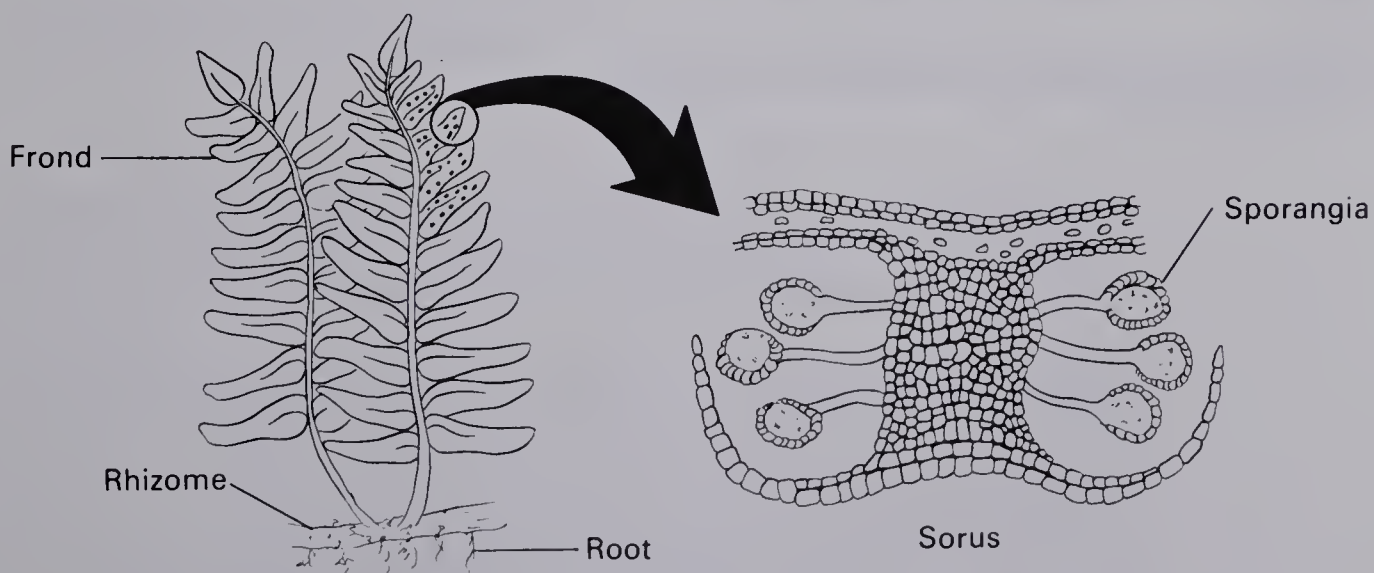
living fern frond with sori	slide and coverslip
prepared slide of fern prothallus	ruler
compound microscope	wetting agent

INTRODUCTION

Ferns belong to a group of plants called pteridophytes (*pterido* means “fern”; *phyte* means “plant”). Ferns are usually found in moist environments. Like bryophytes, they have alternate sporophyte (asexual) and gametophyte (sexual) generations. Both fern generations produce independent, free-living plants. In contrast, in bryophytes only the gametophyte plants can live independently.

The fern sporophyte, with its large and sometimes lacy fronds, is the most familiar of the fern generations. The sporophyte has vascular tissue, which transports water and nutrients to cells far above the ground and provides support. For this reason, fern sporophytes can grow much larger than bryophytes, which lack vascular tissue. In some parts of the world, fern sporophytes grow to tree size.

At certain times of the year, when the fronds are mature, brown spots or lines called sori form on the underside of the fronds. The sori are made up of groups of sporangia, which produce spores. When the spores germinate, they grow into the gametophyte plants.



The gametophyte fern plants do not resemble the sporophytes. The gametophytes are tiny, usually less than 1 cm in diameter, and lack vascular tissue. They grow close to the ground in moist, shaded areas.

Gametophytes reproduce sexually by producing gametes. The zygotes (fertilized eggs) grow into the independent sporophyte plants. The alternation of generations then continues through successive cycles.

PROCEDURE

A. Sporophytes

Examine a fern sporophyte. Note the horizontal stem at the base of the frond(s). This is a rhizome, and is normally located underground. The roots are attached to the rhizome. The fronds, rhizomes, and roots of a sporophyte all have vascular tissue.

1. Vascular tissue transports water and nutrients throughout the plant. What is another function of this tissue, which allows the plants to grow large?
-

Locate the brown sori on the underside of the frond.

2. Draw a portion of the frond showing the distribution of the sori.

Scrape a few sporangia from a sorus onto a clean, dry slide. Do not add water or a coverslip to the slide. Observe the sporangia under low power with a compound microscope. The focused light should produce enough heat to cause the sporangia to split open and release the spores, which look like dust.

3. Draw the closed sporangium and the opened sporangium.

Now add a drop of wetting agent to the slide and cover with a coverslip. Observe the slide under low power and then high power with the compound microscope. Note that the spores are unicellular.

4. Draw one spore.

Spores are released into the air and dispersed by the wind. Those that reach a warm, moist place will germinate. The spores produce algallike filaments called protonemas, which develop into mature gametophytes.

B. Gametophytes

The fern gametophyte plant is a small, heart-shaped structure called a prothallus. On the lower surface of the prothallus are numerous rhizoids, which absorb water and nutrients from the soil. Because the prothallus lacks vascular tissue, water moves through the plant by osmosis. Nutrients move by diffusion.

Examine a prepared slide of a fern prothallus without the aid of a microscope.

5. What is the largest diameter (in mm) of the prothallus?

The fern gametophyte has both male and female gametes on the same plant. The gametes are located on the underside of the prothallus.

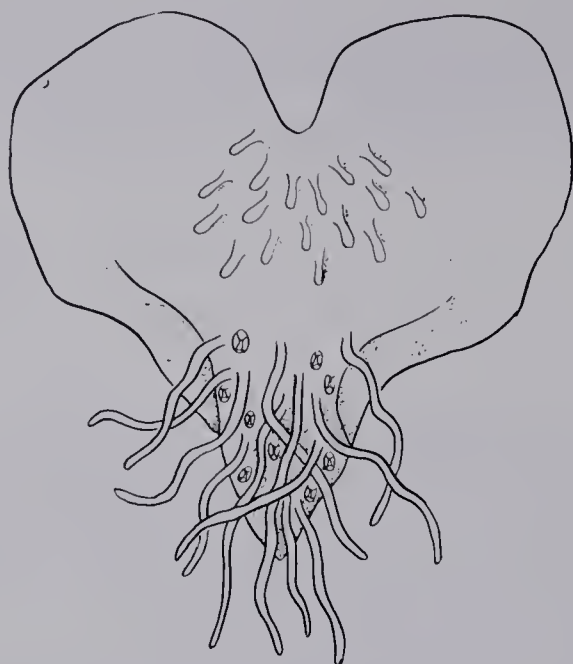
Sperm develop in the male antheridia, and eggs develop in the female archegonia. At maturity, the sperm swim to the archegonia and fertilize the eggs.

6. In what kind of environment must the gametophyte live in order for fertilization to take place?

Examine the prothallus under low power with the compound microscope. Locate the antheridia, which are near the edges of the prothallus. They are round, usually occur in groups, and the sperm inside often appear black.

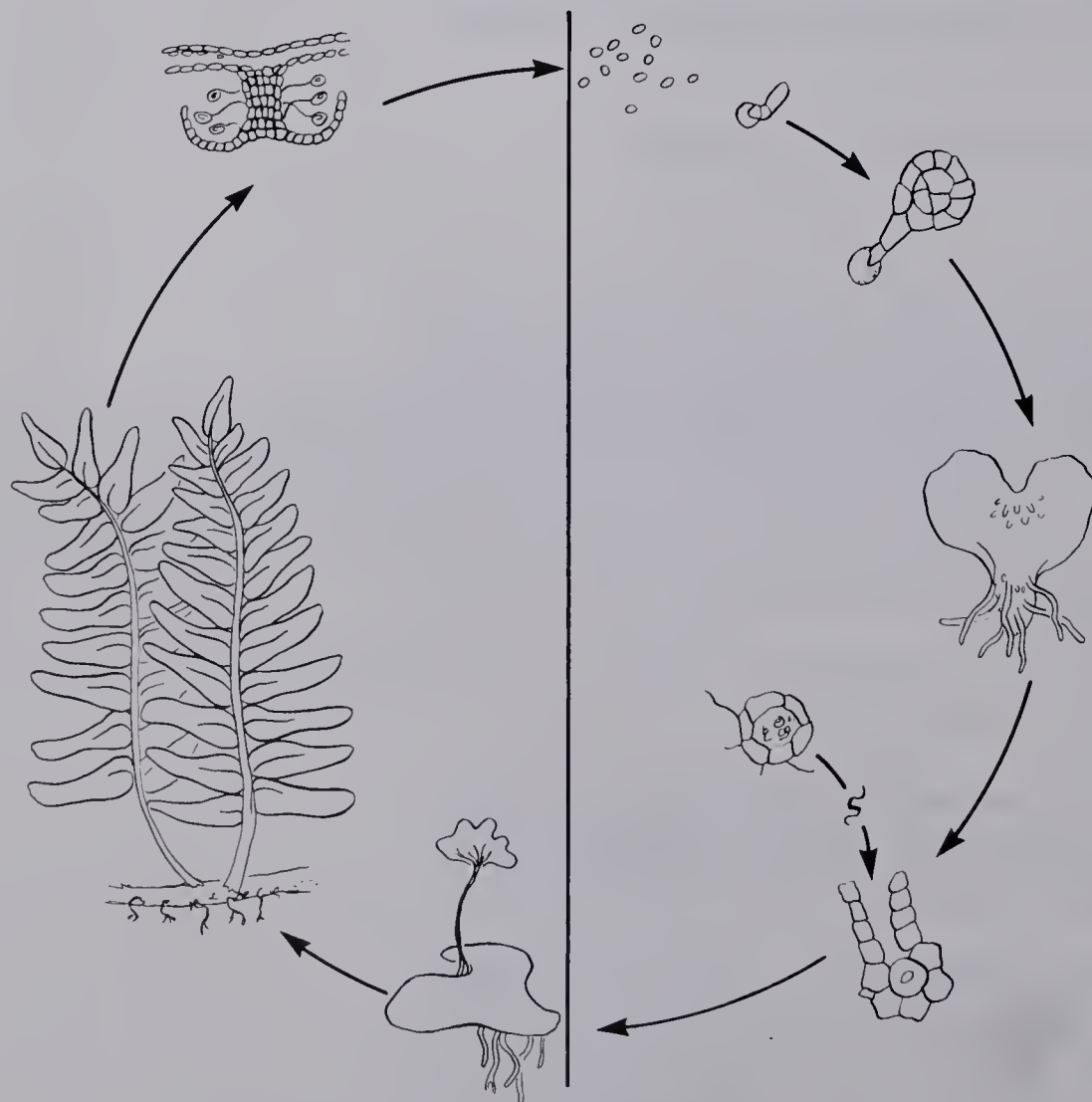
Locating the archegonia is more difficult. They are small vase-shaped structures containing a single egg in the bottom of the "vase." The archegonia are located close to the notch in the prothallus. In order to find them you must focus up and down with the fine adjustment of the microscope. They will appear as fingerlike projections from the surface of the prothallus.

7. Find the archegonia on your prothallus. On the diagram of the prothallus, label the antheridia, archegonia, and rhizoids.



The zygote produced by the gametophyte becomes a young sporophyte plant. The developing sporophyte remains attached to the gametophyte by a stalk for a short time before becoming an independent plant. The gametophyte then disintegrates.

8. On the diagram of the life cycle of a fern, label each structure.



ANALYSIS

9. Why is it advantageous for the antheridia and archegonia to be located on the underside of the prothallus?

10. What structure absorbs water and nutrients in sporophytes? in gametophytes?

11. How are water and nutrients transported throughout the plant in sporophytes? in gametophytes?

12. In the fern, the sporophyte plant is dominant in the sense that it is larger than the gametophyte. Conversely, in the bryophytes, the sporophyte is not the dominant generation. What development in the fern sporophyte makes it the dominant generation? Why?

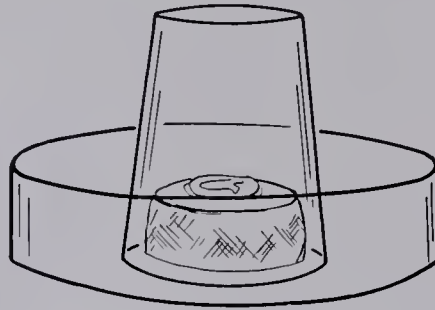
13. How could you determine whether the rhizoids of the prothallus are true roots?

FOLLOW-UP

- Grow fern gametophytes. Soak a peat pellet in water until it is fully expanded—about five minutes. Place the pellet in a culture dish. Collect some fresh spores from the ripe sporangia on a frond (as you did in the lab). Sprinkle the spores on the moist surface of

the peat pellet. Cover the pellet with a clear plastic drinking glass. Pour a small amount of water into the culture dish. Every few days, add enough water to keep the peat moist.

After three weeks, the fern gametophytes should be visible. Keep the glass over the pellet, and watch the gametophytes' growth. When they are fully mature, the fronds of the sporophyte plant will emerge from the prothalli.



- Make a spore print from a fern. Place a fern frond, sporangia-side down, on a piece of white paper. Leave the frond and paper undisturbed for a few days. Then, carefully pick up the frond and observe the spore pattern on the paper.

45 Flower and Seed Structure

PURPOSE

To become familiar with the structure of flowers and seeds.

MATERIALS

gladiolus flower

soaked lima bean seed

dissecting microscope

hand lens

scalpel or razor blade

INTRODUCTION

There are over 200 000 species of flowers in the world. They range in size from microscopic water blossoms to tropical flowers as big as 91 cm across. They range in shape from the familiar star to shapes mimicking insects or birds.

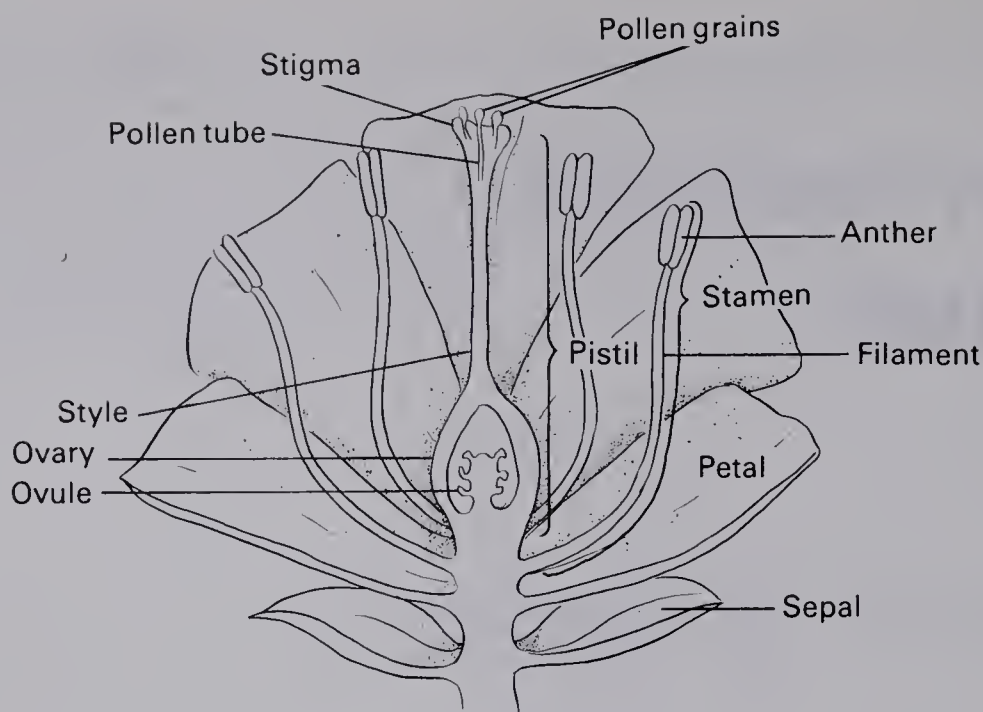
As varied as flowers are, they all play the same role in the plant life cycle. Flowers are the reproductive parts of flowering plants, the angiosperms. Flowers propagate by producing seed-containing fruits. After the fruit ripens, the seeds are released and germinate to produce new plants.

In this lab you will examine the structure of a gladiolus flower and a lima bean seed and learn the functions of the flower and seed parts.

PROCEDURE

A. Flower Structure

There are four basic parts of a flower: petals, sepals, stamens, and pistils. Some flowers do not have all four parts, and are called incomplete flowers. The oak flower, for example, has no petals. Flowers that have all four parts are called complete flowers. On the generalized diagram of a flower, note the structures.



Examine a gladiolus flower, which is complete. The petals are the showy, colorful outer structures. Petals protect the inner flower parts and attract insects and other pollinators.

The sepals are leaflike parts below the petals. In most species they are green, but they can be colored in some species. The sepals enclose the flower when it is a bud and help support the flower when it opens.

1. How many sepals does your gladiolus flower have?

2. How many petals does it have?

Some flowers have both male and female reproductive organs and are known as perfect flowers. Some have only male or only female reproductive organs and are known as imperfect flowers. The gladiolus is a perfect flower.

Gently pull the petals and sepals off the flower. When removing these structures, take care not to destroy the widened base of the flower where they are attached.

Locate the stamens, which look like stalks with swords on their ends. The stamens are the male reproductive parts of the flower. The stalklike part is the filament, and it supports and swordlike anther. The anther produces pollen grains, which contain sperm. Examine the stamens with a hand lens.

3. How many stamens does your gladiolus have?

Locate the pistil, a stalklike structure that widens at the base. This is the female reproductive part of the flower. At the top of the pistil is the stigma (three-pronged in the gladiolus). The sticky surface of the stigma is an adaptation for collecting pollen.

Pollen is transferred from the anther to the stigma by insects and other pollinators, which are attracted by the petals. In flowers without petals, pollen is often transferred by wind.

The pollen germinates in the stigma, developing pollen tubes that grow down the slender style to the vase-shaped ovary. Sperm travel through the pollen tube to the ovary. Only pollen from a flower of the same species will reach the ovary. Examine the pistil with a hand lens.

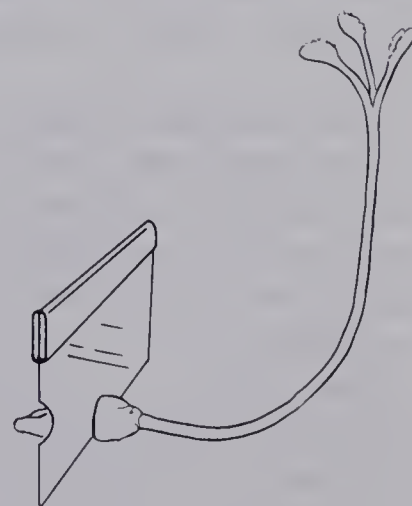
4. Draw one of each of the parts of your gladiolus: petal, sepal, stamen (including the filament and anther), and pistil (including the stigma, style, and ovary). Label the parts.

The number of flower parts indicates whether a flower is from a monocot or dicot plant. Most structures of a monocot flower usually occur in multiples of three—for example, three, six, or nine petals. The structures of a dicot flower usually occur in multiples of four or five.

5. Is the gladiolus a monocot or dicot?

Many ovaries are divided into chambers called carpels. Inside the carpels are numerous ovules, each of which contains an egg. After the eggs are fertilized, the ovules will develop into seeds.

Using a scalpel or razor blade, cut the ovary in half crosswise, as illustrated. Examine the cut surface under the dissecting microscope. Locate the carpels and the white dotlike ovules.



Caution: Cut down on a hard surface, away from your fingers.

6. Draw a cross section of the ovary, showing the number of chambers and the placement of the ovules.

Actually, two sperm are involved in producing a seed in a flowering plant. One sperm fertilizes the egg, which develops into the embryo. The nucleus of a second sperm unites with two nuclei of a cell in the middle of the ovule. This second fertilization forms a tissue called endosperm. Endosperm provides food for the developing embryo. Since two sperm are essential in producing a seed, flowering plants are said to have double fertilization.

B. Seed Structure

After fertilization, the embryo develops into a tiny plant inside the seed. The seed is a mature ovule. As the ovules inside an ovary mature into seeds, the ovary develops into a fruit. A fruit is a ripened ovary containing seeds.

In the lima bean plant, the fruit is the bean pod and the seeds are the beans inside the pod. During their development, the bean embryos used up all the endosperm. Unlike lima beans, some mature seeds such as corn retain some endosperm. Popped corn is exploded endosperm.

Examine the outside of the bean seed. Note the scar, called the hilum, where the seed was attached to the pod. Near the hilum is a small hole, called the micropyle, where the pollen tube entered the ovule.

With a scalpel or razor blade, carefully remove the outer covering of the bean, the seed coat. Open the seed into two halves longitudinally. The halves are called cotyledons. Below the point of attachment of the cotyledons, you will see the little pair of leaves and the stem. The embryo consists of three parts: the cotyledons, the leaves, and the stem.

The number of cotyledons in a seed indicates whether the seed is from a monocot or a dicot plant. A monocot has one cotyledon and a dicot has two cotyledons.

7. Is the lima bean seed a monocot or a dicot?

8. On the diagrams of a closed and open bean seed, label the hilum, micropyle, cotyledons, leaves, and stem.



ANALYSIS

9. List the functions of the following flower parts and note whether they are part of the pistil or stamen.

anther _____

filament _____

ovary _____

ovule _____

pollen _____

stigma _____

style _____

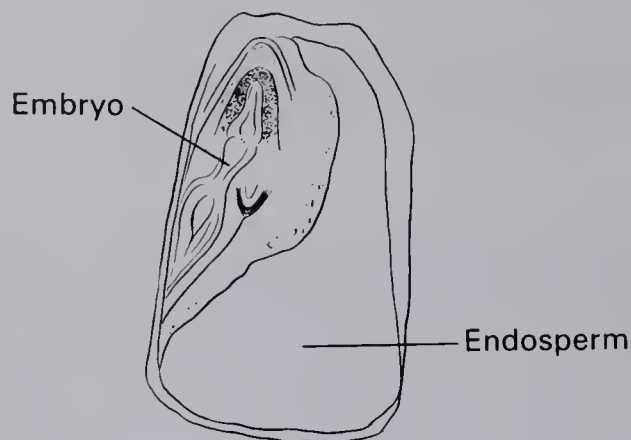
10. The number of pollen grains produced by one anther is much greater than the number of ovules in one ovary. How is the high production of pollen an advantage to a plant?

11. Review the descriptions of complete and incomplete flowers, and perfect and imperfect flowers. a) Can an imperfect flower be complete? Why or why not? b) Can an incomplete flower be perfect? Why or why not?

12. If one ovary contains nine ovules, how many fruits and seeds will develop after fertilization?

13. The lima bean seeds were soaked in water before class so that it would be easier to remove the seed coat and open the halves. Through what structure could the seeds have absorbed the water?

14. Is the corn seed pictured a monocot or a dicot? Why?



15. How do people's existence depend on flowering plants?

FOLLOW-UP

Examine the leaves of the lima bean embryo under the dissecting microscope. Draw the miniature leaf, showing the veins.

46 Seed Germination

PURPOSE

To investigate the conditions under which seeds germinate.

MATERIALS

10 dry corn or bean seeds	paper towels
20 soaked corn or bean seeds	parafilm or aluminum foil
boiled water cooled to room temperature	refrigerator or ice chest
6 beakers, 250 mL	rubber bands
drinking straw	

INTRODUCTION

A seed contains an embryo (an immature plant), which may germinate, or sprout, and grow into a mature plant. Germination occurs only when certain environmental conditions are met.

If conditions are unfavorable, seeds remain dormant (alive, but not growing). The outer seed coat protects the embryo. Consequently, seeds often can survive conditions that would kill growing plants. Dormant weed seeds can remain viable, capable of growing, for thirty years.

Germination is affected by temperature, oxygen, and moisture. In this lab you will investigate whether all three factors are necessary for germination to occur.

The class will test both corn and bean seeds in order to investigate how different kinds of seeds respond to the same factors. Half the class will use corn seeds in all three experiments, and the other half will use bean seeds in all three experiments. Be certain to use the same kind of seeds in each experiment.

PROCEDURE

1. Temperature

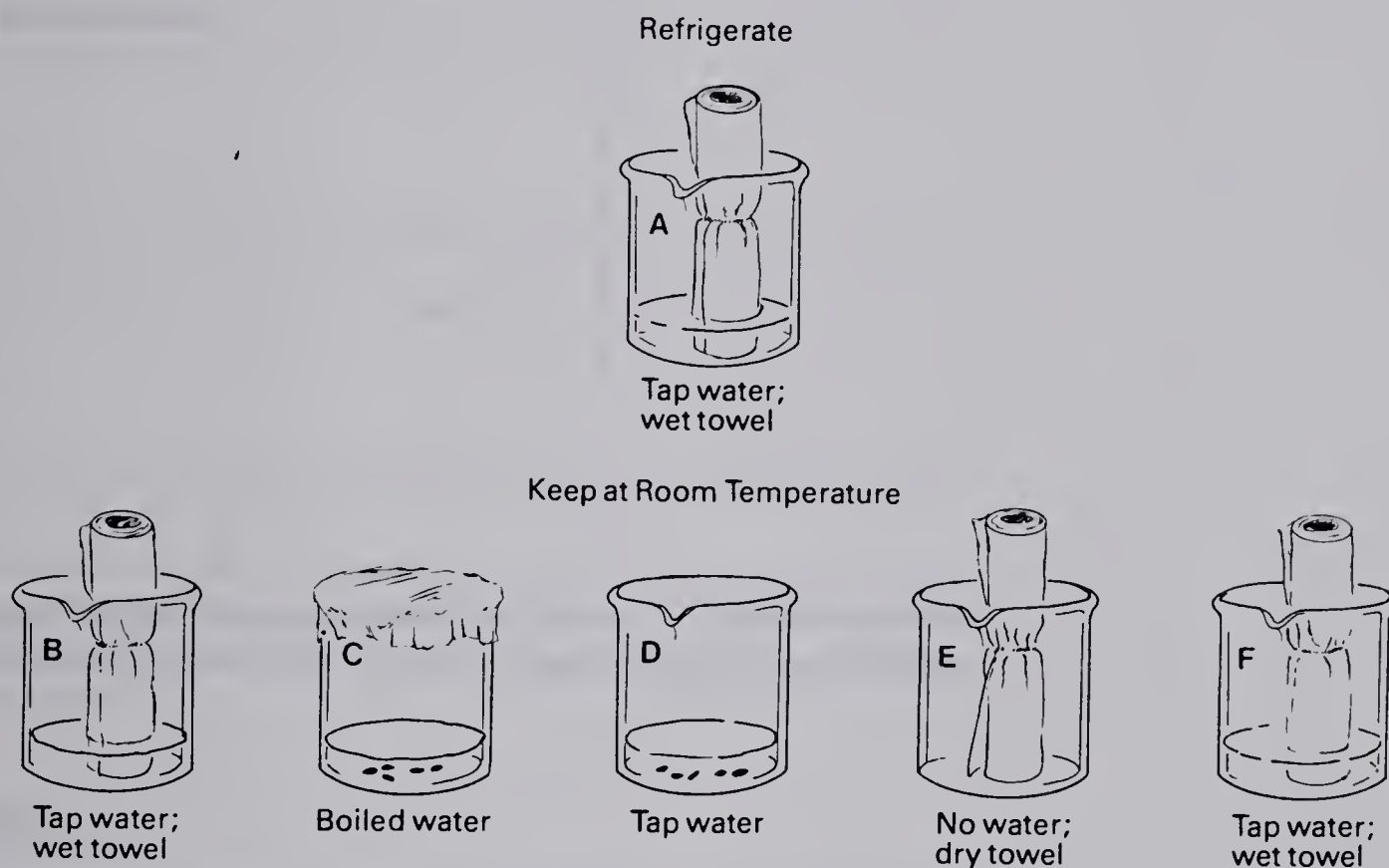
Obtain ten soaked seeds. Wrap five of the seeds in a wet paper towel and secure it with a rubber band. Place the towel on end in a beaker. Add enough tap water to the beaker to wet the towel but not immerse the seeds. Label the beaker A and put it in a refrigerator.

Prepare the other five seeds in the same manner. Label the beaker *B* and put it on a shelf or counter at room temperature.

Check the beakers daily for five days to see whether the water has evaporated. Add more water if necessary to keep the ends of the towels wet. On the fifth day, unwrap the seeds and see how many have sprouted. Record the data on the data chart.

B. Oxygen

Obtain ten soaked seeds. Place five of the seeds in a beaker and add enough boiled water to cover the seeds. Cap the beaker tightly with parafilm or aluminum foil. Label the beaker *C*.



Place the other five seeds in a second beaker and add enough room temperature tap water to cover the seeds. Blow air through the water with a drinking straw for one minute. Do not cover this beaker; label it *D*. Place both beakers with beaker *B* on the counter.

Blow air in the water in beaker *D* each day. Add water to the beakers if necessary to keep the seeds covered. Be sure to use boiled water in beaker *C*. On the fifth day, check the seeds and record the data on the chart.

C. Moisture

Obtain ten dry seeds. Wrap five of the seeds in a dry paper towel. Secure the paper towel with a rubber band and place it on its end in a dry beaker. Label the beaker *E*.

Wrap the other five dry seeds in a wet paper towel and secure it with a rubber band. Place the towel on its end in a beaker. Add enough tap water to wet the towel but not immerse the seeds. Label the beaker *F*. Place both beakers with beakers *B*, *C*, and *D*.

Check beaker *F* daily to see if more water is necessary. On the fifth day unwrap the seeds and record the data on the chart.

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF CHEMISTRY
JANUARY 1964

REPORT OF THE
COMMISSION ON THE
FUTURE OF THE DEPARTMENT



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DEPARTMENT OF CHEMISTRY
JANUARY 1964

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DEPARTMENT OF CHEMISTRY
JANUARY 1964

Name _____ Date _____

	Percent Germination	
	Corn	Bean
Temperature		
A (cold)		
B (room temperature)		
Oxygen		
C (low)		
D (high)		
Moisture		
E (dry)		
F (wet)		

Note: Percent germination is calculated as follows: Divide the number of seeds germinated by the total number of seeds in the group. Multiply the answer by 100.

ANALYSIS

1. What differences, if any, were there between corn and bean seed germination in the three experiments?

2. According to the data, which of the environmental conditions tested are necessary for the germination of corn and bean seeds?

3. Which of the three conditions tested would most likely be a limiting factor in the germination of seeds of desert plants?

4. Which condition would be most limiting in aquatic plant seeds?

5. What other environmental conditions do you think would affect the germination of certain seeds?

47 Development in Frogs

PURPOSE

To observe the sequence of frog development from egg to tadpole.

MATERIALS

fertilized frog eggs

boiled lettuce leaves

pond water or aerated tap water

dissecting microscope

eyedropper

small beaker or finger bowl

forceps

scissors

INTRODUCTION

Every spring the secretion of pituitary hormone increases in male and female frogs. Under that influence, the frogs gather at ponds for the annual courtship ritual that results in mating. The mating pairs simultaneously shed eggs and sperm into the water, where fertilization takes place. Within a few hours the zygote begins dividing, and within two weeks it has become a tadpole.

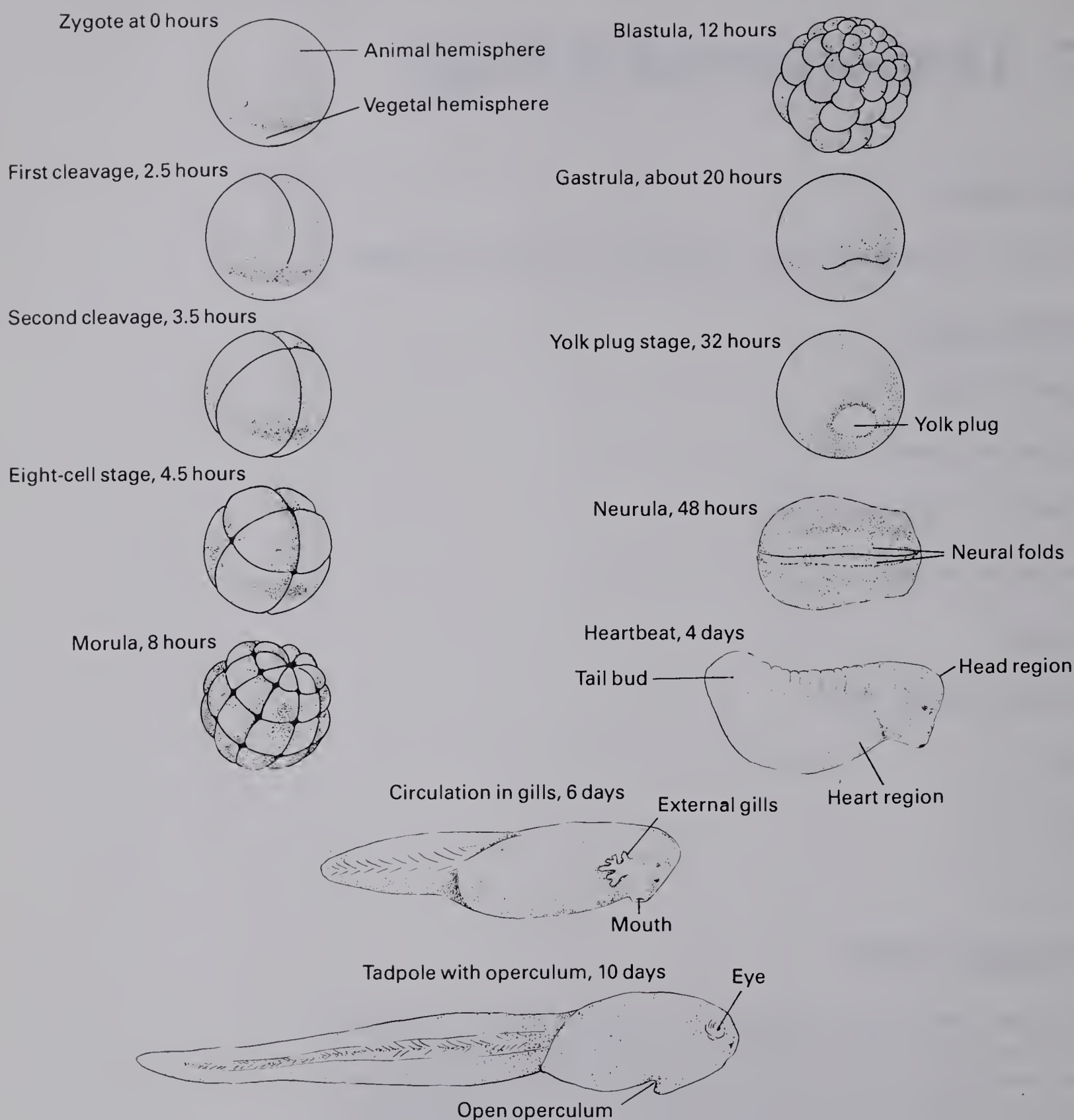
Between egg and tadpole is a series of complex events. In this lab you will observe some of these stages of development.

PROCEDURE

A. Stages of Development

Familiarize yourself with the following stages of development before observing a frog egg in part B.

The zygote is divided into two regions. The dark animal hemisphere, which is on top, develops into the embryonic frog. The lighter-colored vegetal hemisphere, which is on the bottom, contains yolk.



1. What is the yolk used for?

After fertilization, the zygote undergoes a series of divisions called cleavage. The first two cleavages, into two and then four cells, are perpendicular to each other and cut through both hemispheres. The next cleavage, into eight cells, is crosswise and well inside the animal hemisphere. More of the succeeding cleavages are inside the animal than the vegetal hemisphere.

As cleavage continues, the embryo begins to resemble a raspberry. This stage is called the morula. Then a hollow ball of 64 cells, called the blastula, is formed.

The next stage, the gastrula, is formed as cells on the surface of the blastula migrate inside the zygote. This leaves a yolk plug protruding from the inner layer.

The embryo now elongates into the neurula stage. The neural plate (cells that will form part of the nervous system) appears on the dorsal surface. The neural plate soon is enclosed by a pair of ridges called the neural folds.

2. What structures will arise from the neural folds?

As development continues, the embryo looks more and more like a tadpole. It continues to elongate. Gills, a mouth, and a tail bud (the region that forms the tail) appear. Internally, the nervous system, muscles, and other systems are developing. Before the tadpole is formed, the external gills are lost and the internal gills are covered with a fold of skin called the operculum.

About ten days after fertilization, the embryo becomes a tadpole. It will be at least two or three months more before the tadpole metamorphoses (changes in form) into an adult frog. The tail, gills, and other organs are reabsorbed during metamorphosis.

B. Observing a Zygote

The zygote you will observe came from a biological supply house, where it was artificially fertilized. Your teacher will tell you how many days old the zygote is. Because the egg was kept in cold storage, its development was slowed. To determine the stage of development of your zygote, you will have to consult the descriptions and illustrations in part A.

Fill the beaker or finger bowl with pond water to a depth of about 1 cm. Using forceps and scissors, carefully cut away an egg from the large egg mass. Place the egg in the pond water, and examine it under the dissecting microscope.

3. At what stage of development is your zygote?

Observe the zygote throughout the lab period. At each stage of development you witness, write the date, time, and stage on the data chart. Make a sketch of the embryo as it appears at each stage.

Embryos live on their yolk supply until they hatch out of the eggs and become tadpoles. At about this time the eyes, mouth, and external gills appear. This occurs about six days after fertilization.

When the tadpoles hatch, you should begin feeding them. Shred about 1 cm² of boiled lettuce leaf and place it in the pond water. At the end of the lab, remove any uneaten food. Also, remove the old pond water with an eyedropper and replace it with fresh pond water.

At the end of the period, put your embryo (or tadpole) in the place designated by the teacher. In the next lab period you will continue to

Frog Embryo Development

<i>Date</i>	<i>Time</i>	<i>Stage of Development</i>	<i>Sketch of Embryo</i>

observe the embryo and fill in the chart. Remember—embryo development will continue between labs.

At the beginning of the second lab period, determine the stage of development of your embryo.

4. Which stages, if any, occurred between lab periods?

If the embryo has hatched, remember to feed it and change the water.

ANALYSIS

5. What are the main stages of embryonic development for frogs (and other amphibians)?

6. In birds and mammals, amniotic fluid protects an embryo. What protects a frog embryo during development?

7. How do the number of frog eggs and the color of the eggs help ensure survival of at least some tadpoles?

8. How are tadpoles evidence for a common origin of fish and amphibians?

FOLLOW-UP

Keep the tadpole in an aquarium until it becomes a frog and observe the changes. Remember to feed the tadpole and change the water often. When it becomes a frog, change its diet to small bits of raw liver.

Before a tadpole completes its metamorphosis into a frog, it must undergo all the changes necessary to transform an aquatic animal into a terrestrial one. In nature, most of these changes occur during the summer, and the tadpole hibernates over the winter before becoming an adult frog. Under laboratory conditions, metamorphosis may be complete in two or three months.

During metamorphosis, the tadpole becomes much larger. Its internal gills carry on respiration, but the animal also begins to grow the lungs it will use as an adult. The tail is gradually absorbed, limbs appear, and the body changes to the frog shape. The animal also changes from an herbivore to a carnivore.

The adult frog should not be released into the local environment. It may be placed in a suitable terrarium in your school or home.

48 Smooth and Wrinkled Peas

PURPOSE

To examine the genetic differences in smooth and wrinkled peas.

MATERIALS

Part A:

- | | |
|----------------------|------------------------|
| 20 dried smooth peas | 20 dried wrinkled peas |
| balance | 2 small beakers |
| paper towels | wax pencil |

Part B:

- | | |
|---------------------|--------------------------|
| 1 soaked smooth pea | 1 soaked wrinkled pea |
| compound microscope | 2 slides with coverslips |
| razor blade | wax pencil |

Part C:

- | | |
|--|---|
| 5 dried smooth peas | wax pencil |
| distilled water | 5 dried wrinkled peas |
| petri dish with glucose-1-phosphate agar | iodine indicator solution |
| eyedropper | funnel |
| mortar and pestle | 2 test tubes with stoppers (or aluminum foil) |

INTRODUCTION

Contrary to the popular saying, all peas in a pod are not always alike. When dried, peas from the same pod may be either smooth or wrinkled. How can this be? The environmental conditions in the pod were the same for all the peas, so the cause of the difference is not environmental. The cause must be genetic.

Let this be the working hypothesis: The difference in the genetic makeup of the peas determines whether they will be smooth or wrinkled.

To test the hypothesis you will perform three experiments. You will look for structural, microscopic, and biochemical differences between smooth and wrinkled peas of the same species.

PROCEDURE

A. Structural Differences

Obtain 20 dried smooth peas and 20 dried wrinkled peas. Weigh the peas on a balance and record the data on the data chart.

Place the wrinkled seed in one beaker and the dried seeds in another. Fill the beakers three-quarters full of water. Using a wax pencil, label the beakers with the kind of peas inside and your name.

	<i>Dry Weight</i>	<i>Wet Weight</i>	<i>Net Change</i>	<i>Percent Change</i>
Smooth				
Wrinkled				

After 24 hours, remove the peas from the water. Blot them dry with a paper towel. Weigh them again and record the data on the chart.

Make the calculations for the last two columns of the chart. To compute the percent change use this equation:

$$\frac{\text{Wet weight} - \text{dry weight} \times 100}{\text{dry weight}}$$

1. After 24 hours, is there any difference in the roundness of the smooth and wrinkled peas?

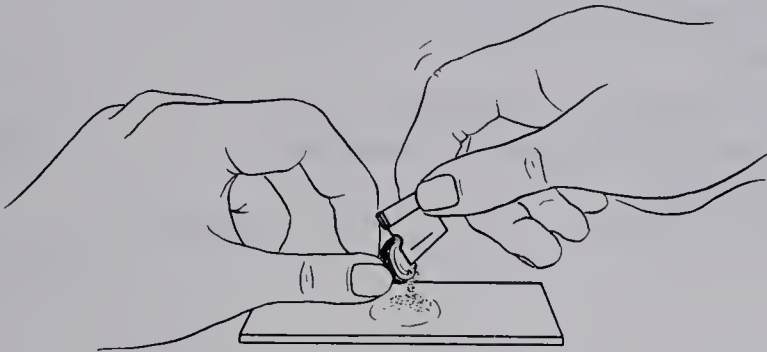
2. Which peas gained the most weight?

3. Assume that the weight change is due to water absorption. If you were to dry out the peas, which would lose the greatest percentage of water?

4. On the basis of this experiment, why do you think some peas wrinkle and remain smooth when they are dried?

B. Microscopic Differences

Obtain one soaked, smooth pea, one soaked, wrinkled pea, and two slides. With a wax pencil, label one slide *S* and the other *W*. Put a drop of water on each slide.



With a razor blade, cut the smooth pea in half. Scrape the cut surface allowing the scrapings to fall into the water drop on the *S* slide. Mix the water and scrapings, and cover with a coverslip.

Carefully wash the razor blade in water. Prepare the wrinkled pea and *W* slide as you did the smooth pea and *S* slide.

Observe each slide under low power with the compound microscope. Note the granules—these are starch grains.

Caution: Cut down on a hard surface, away from your fingers.

5. Describe the difference in appearance of the starch grains from the smooth and wrinkled peas.

6. Which kind of starch grains must have the greatest surface area per unit volume?

7. Which kind of starch grains can absorb more water? Why?

C. Biochemical Differences

Obtain five dried smooth and five dried wrinkled peas. You will extract the starch-forming enzymes from both types of seed.

Crush the smooth peas with a pair of pliers, letting the crushed peas fall into a mortar. Grind the peas in the mortar with a pestle. Add

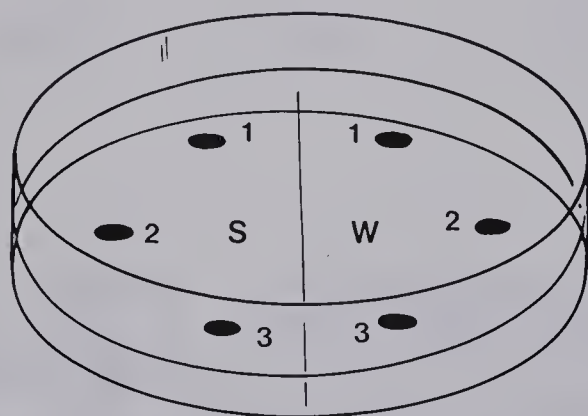
10 mL of distilled water to the mortar and continue to grind the peas until a smooth watery paste is formed. Using a funnel, transfer the contents of the mortar to a test tube. Label the tube *S*.

Wash the pliers, mortar, and pestle. Repeat the procedure with the wrinkled peas; label the tube *W*. With a wax pencil, write your name, the date, and your class period on the tubes. Stopper both tubes and put them in the refrigerator for 24 hours.

When you remove the tubes from the refrigerator, try not to stir the contents. If the liquid on top is not clear, filter it before you proceed.

Obtain a petri dish containing glucose-1-phosphate agar. With a wax pencil, draw a line across the bottom of the dish to divide it in half. Write an *S* on one half and a *W* on the other.

With an eyedropper, place three drops of the clear liquid from the *S* test tube on the *S* side of the petri dish. Clean the dropper thoroughly. Place three drops from the *W* tube on the *W* side.



Add one drop of iodine to the enzyme spots in numbered order. Allow 15 minutes between drops.

The enzymes from the test tubes should change the glucose in the agar to starch. You have seen that the starch grains in the two types of peas are different. Are the enzymes producing the starches different?

To test for starch production on the agar, you will use iodine indicator solution. Iodine turns blue-black in the presence of starch. Place one drop of iodine on *one* spot of the *S* slide and *one* spot of the *W* slide. After 15 minutes, add iodine to a new spot on each side. After another 15 minutes, add iodine to the third spots.

8. Do you see any difference between the actions of the two enzymes? If so, what?

9. What do your results indicate about the starch-making process in smooth and wrinkled peas?

ANALYSIS

10. List all the differences you observed between smooth and wrinkled peas.

11. Which enzyme produces more starch grains at a faster rate? Which starch grains have more surface area per unit volume?

12. How could the difference in number and structure of starch grains in the two kinds of peas explain the difference in water absorption?

13. Do the experiments support the hypothesis in the introduction that the difference in the genetic makeup of the peas determine whether they will be smooth or wrinkled? Explain why or why not.

49 Probability

PURPOSE

To understand the fundamentals of probability and learn how to use the product rule and the chi-square statistical method.

MATERIALS

2 coins

pencil

INTRODUCTION

Take a chance! If 200 chances to win a guitar were sold and you bought 10, what would be the probability that you would win? Probability is expressed as a ratio: the number of times something occurs (10) over the total number of possible occurrences (200). So, you can calculate the probability of winning by dividing 10 by 200. In this case, the probability (p) would be $p = \frac{1}{20}$, or .05, or 5 percent. Probability is a numerical expression of the likelihood that an event will occur.

PROCEDURE

A. Sample Size

When you toss a coin, what is the probability that it will land with the head side up? The answer is $\frac{1}{2}$, or .5, or 50 percent. In 10 throws you would expect to get 5 heads and 5 tails.

When you actually throw the coin, you may not get a 5-heads:5-tails ratio. The difference between what you expect according to probability and what happens is called the deviation. For example, if you get 6 heads and 4 tails your deviation is 2 (1 from the expected 5 heads plus 1 from the expected 5 tails).

Deviation is normally expressed as a percent. The percent deviation is calculated as follows:

$$\text{percent deviation} = \frac{\text{deviation}}{\text{total number of occurrences}}$$

$$= \frac{2}{10} = .2 = 20 \text{ percent}$$

1. Calculate the percent deviation for 3 heads and 7 tails out of 10 throws.

2. Toss a coin 10 times and record the results on the chart. Repeat the process for 9 more trials (of 10 throws each), making 100 throws in all. Total the number of heads and of tails, and calculate the percent deviation for the entire 100 throws.

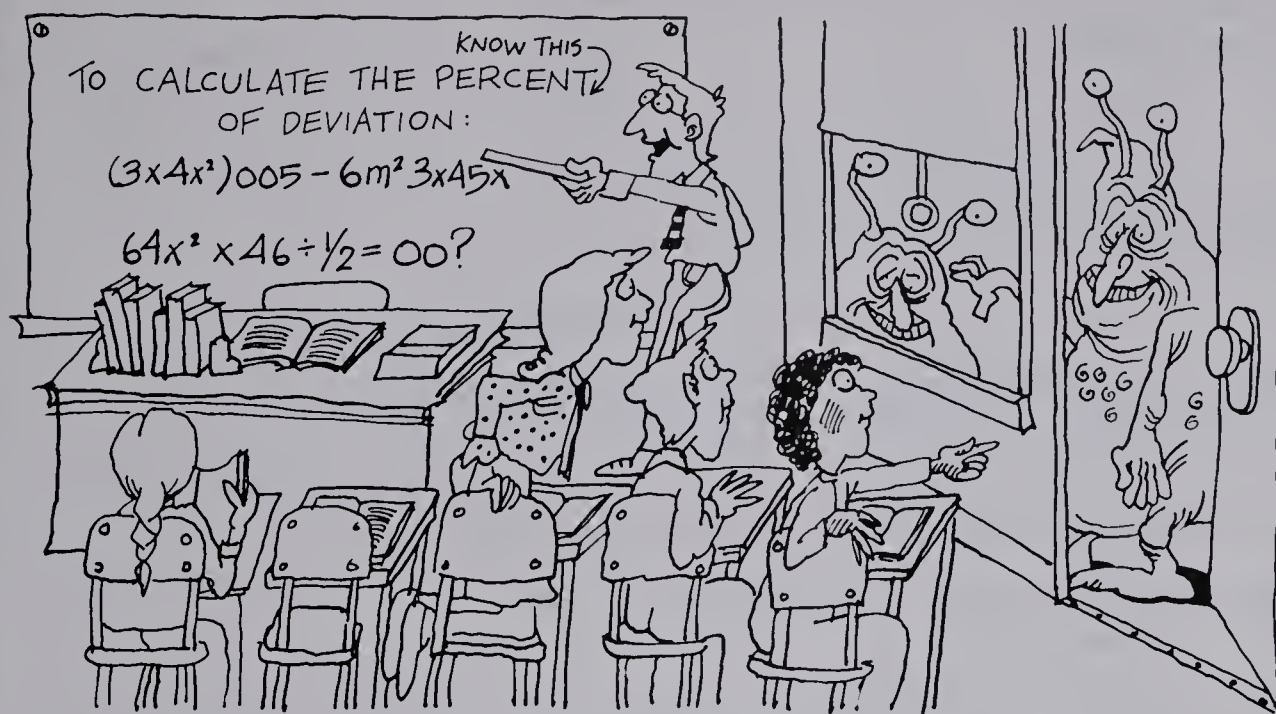
<i>Trial (10 throws each)</i>	<i>Number of Heads</i>	<i>Number of Tails</i>	<i>Deviation (percent)</i>
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Totals _____

Percent deviation for 100 throws _____

3. How does the deviation for a sample size of 10 throws (trials 1-10 compare to the deviation for a sample size of 100 throws (1-10 totals)?

4. What does the answer to question 3 tell you about the relationship between sample size and probability?



"I don't know about you, but I'd call that a deviation."

B. The Product Rule

The product rule states the probability that two independent events will occur simultaneously. Independent events are events that do not influence one another. For example, if you roll two dice, the number that comes up on one does not influence the number on the other.

In this section you will use coins to discover the product rule of probability.

5. Flip 2 coins 100 times. Tally the results on the chart.

	2 heads	1 head, 1 tail	2 tails
Totals			

6. What is the nearest whole-number ratio of the results (two heads: one head and one tail:two tails)? 1:1:1; 2:1:1; 1:2:1; 1:1:2; or some other?

The probability of getting heads on the first coin is $\frac{1}{2}$, or .5; and the probability of getting heads on the second coin also is $\frac{1}{2}$. You can determine the probability that both coins will come up heads by multiplying the individual probabilities.

$$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}, \text{ or } .25$$

7. What is the probability of getting two tails on two coins?

8. What is the probability of getting a head on the first coin and a tail on the second coin?

9. What is the probability of getting a tail on the first coin and a head on the second coin?

Note that there are two ways of getting 1 head and 1 tail—the head first or the tail first. You can find the probability of getting 1 head and 1 tail by adding the two individual probabilities.

First Coin	Second Coin	
head	tail	$p = \frac{1}{4}$
tail	head	$p = \frac{1}{4}$
		$\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$
		$p = \frac{1}{2}, \text{ or } .50$

To summarize, the probability of getting the different combinations of the two coins is:

2 heads	$p = \frac{1}{4}, \text{ or } .25$
1 head, 1 tail	$p = \frac{1}{2}, \text{ or } .50$
2 tails	$p = \frac{1}{4}, \text{ or } .25$

10. Refer to the data for your 100 throws of the two coins and calculate the percent deviation from the expected probability. First calculate the number of occurrences of each combination expected in 100 throws. Then calculate the deviation between your results and the number of occurrences.

11. Complete the following statement:

The Product Rule of Probability: To calculate the probability that two independent events will occur simultaneously, you

C. The Chi-square Statistical Method

If you throw a die, what is the probability of a 4 coming up? Because there are 6 sides on the die, the probability is 1 in 6, or .167, or 16.7 percent. In 60 rolls, you would expect the 4 to come up 10 times. If the 4 came up 9 times, you would probably think that the deviation was due to chance.

However, what if the 4 came up 40 times in the 60 throws? Because the deviation is so great, you might think that the die was “loaded”—that the deviation was not due to chance. The chi-square is a mathematical tool designed to show the probability that chance alone is operating in a situation.

Chi is a Greek letter that looks like an X. Chi-square is normally written “ χ^2 .” It is derived by this equation:

$$\chi^2 = \sum \frac{(\text{expected outcome} - \text{observed outcome})^2}{\text{expected outcome}}$$

In the equation, Σ means to sum the value obtained for each data category. A “category” refers to the possible things that can occur. For example, when you flip a coin you can get a head or a tail, so there are 2 data categories. When you try to roll a 4 on a die, you either roll the 4 or another number (1, 2, 3, 5, or 6). In this case the 2 categories are “4” and “other numbers.” (The number of data categories can be higher, depending on the situation.)

In the die example for 60 throws, we expected 10 fours and 50 other numbers (1, 2, 3, 5, or 6). We observed 9 fours and 51 others. In using the chi-square, add together the value obtained for the 4-category and the value obtained for the other-number-category:

$$\begin{aligned}\chi^2 &= \frac{(10 - 9)^2}{10} + \frac{(50 - 51)^2}{50} \\ &= \frac{1^2}{10} + \frac{-1^2}{50} \\ &= .1 + .02 \\ \chi^2 &= .12\end{aligned}$$

Once a chi-square value is determined it must be interpreted with the use of the chi-square table (on the following page).

Because there are 2 data categories, look for the chi-square value on the line marked “For 2 data categories.” The chi-square value of .12 falls between the values listed in column 2 (.004) and column 3 (.455).

	<i>Chi-square Values</i>							
	.001	.004	.455	1.074	1.642	2.706	3.841	6.635
For 2 data categories								
For 3 data categories	.020	.103	1.386	2.408	3.219	4.605	5.991	9.210
For 4 data categories	.115	.352	2.366	3.665	4.642	6.251	7.815	11.341
Probability that chance alone is responsible for the deviation	99%	95%	50%	30%	20%	10%	5%	1%

The percent probability that chance alone is operating—the p-value—is shown at the bottom of each column. In column 2 the figure is 95 percent and in column 3 it is 50 percent. So the chi-square value of .12 means that the probability is between 50 percent and 95 percent that chance alone will account for this much deviation. In other words, if 4 comes up 9 times in 60 rolls, the deviation is likely due to chance.

If the p-value turns out to be less than 5 percent (chi-square value higher than 3.841), something other than chance is thought to be involved. In the example of 4 coming up 40 times in 60 rolls, the chi-square value is 108. One would suspect that the die was loaded.

12. Calculate the chi-square value and determine the p-value for the following problem. Show your work.

In 100 throws of a coin, you get 45 heads and 55 tails.

ANALYSIS

13. This problem has three data categories. The chi-square value you should get is 2.92. When you get the correct chi-square value, determine the p-value. Show your work.

In 200 throws of two coins, you get 45 heads, 112 heads/tails, 45 tails.

14. A single coin is tossed 50 times. Heads come up 20 times and tails 30 times. What is the probability that chance alone is responsible for this deviation?

50 *Drosophila* Inheritance

PURPOSE

To demonstrate heredity patterns in fruit flies.

MATERIALS

Per team of 2-4:

2 *Drosophila* cultures (wild-type and recessive mutant)

dissecting microscope

two file cards

ether in dropper bottle

gummed labels

adhesive tape

hand lens

camel's hair brush

morgue (capped bottle half-filled with vegetable oil)

2 culture bottles

etherizer bottle with cork and cotton plug

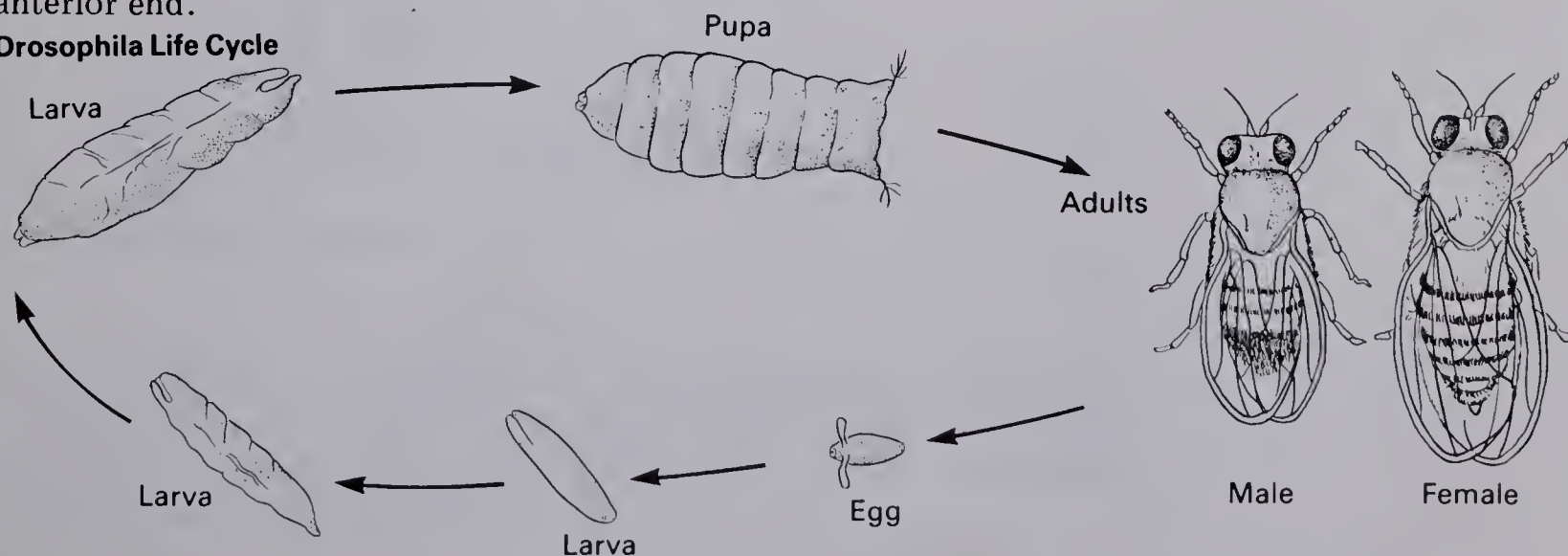
reetherizer (petri dish with cotton taped inside)

INTRODUCTION

The common fruit fly, *Drosophila melanogaster*, is a useful experimental animal. This small insect has many traits that are easily observed without magnification. These traits include eye color, wing size, and body color. The fruit fly has only one pair of wings; most other kinds of insects have two pairs of wings.

When the fruit flies reproduce, a new generation appears in about two weeks. The life cycle includes the stages of egg, larva, pupa, and adult. The larvae are small white maggots with small black spots on the anterior end.

Drosophila Life Cycle



In this lab you will examine two strains of fruit flies, and cross the two strains.

PROCEDURE

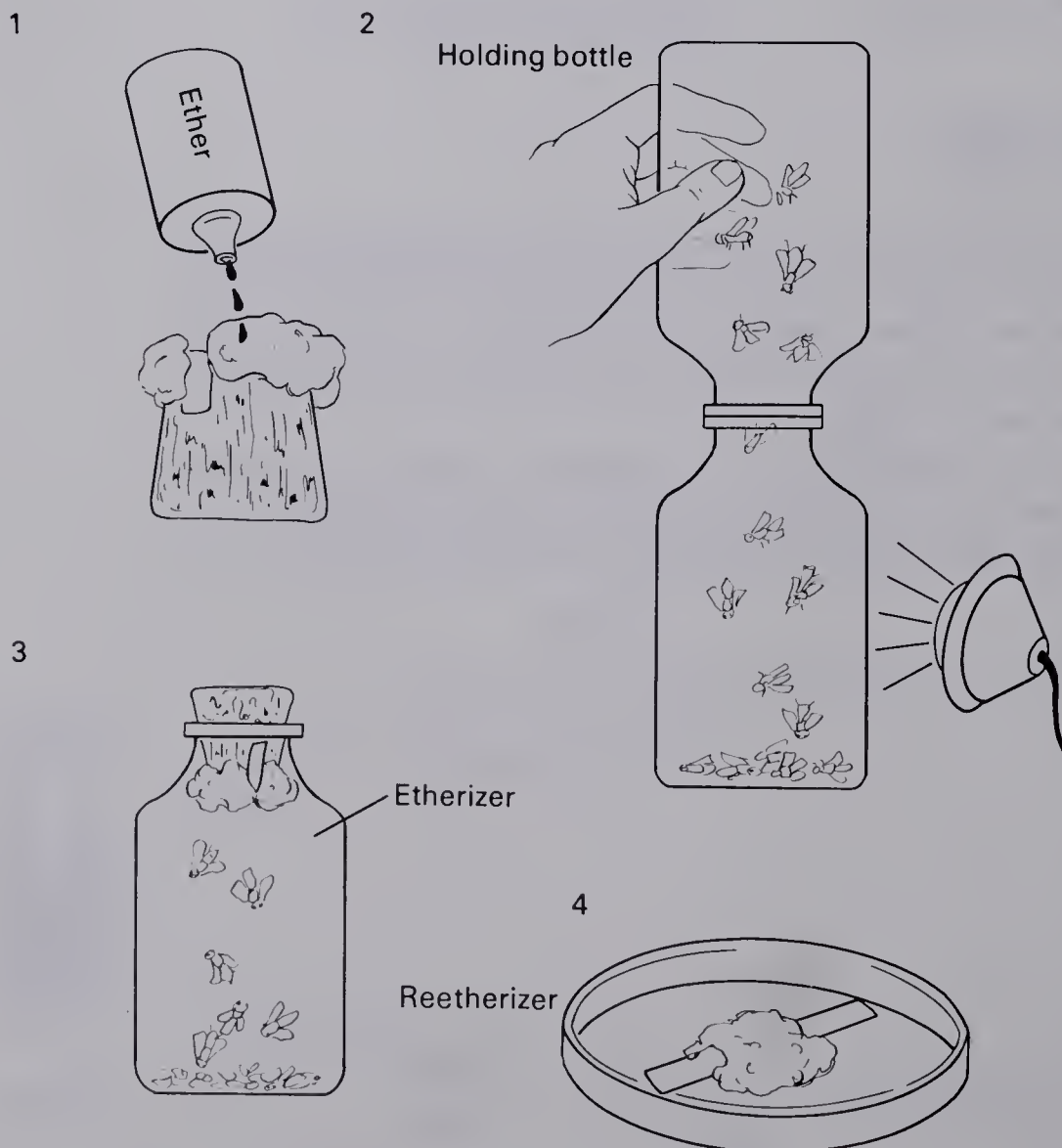
A. Preparing *Drosophila*

Draw a line across the center of a file card, lengthwise. Draw the symbol for male, ♂, in one half and the symbol for female, ♀, in the other half. Write your name on the card.

Your Name	
	♂
	♀

Obtain the following: a holding bottle of wild-type flies, a dropper bottle of ether, a culture bottle containing *Drosophila* medium, an etherizer bottle, and a reetherizer (petri dish with cotton).

Prepare the etherizer bottle as follows. Tape the cotton to the cork, and drop several drops of ether on the cotton. Set the cork and cotton aside.



Transfer the flies to the etherizer bottle as follows. Gently tap or shake the flies to the bottom of the holding bottle. Remove the stopper from the holding bottle and immediately place the etherizer bottle over the holding bottle. Keeping the open ends of the two bottles together, carefully invert the bottles so that the etherizer bottle is on the bottom. Position the etherizer bottle near a desk lamp as in the illustration. The flies will move toward the light. When all the flies are in the etherizer bottle, quickly insert the cork.

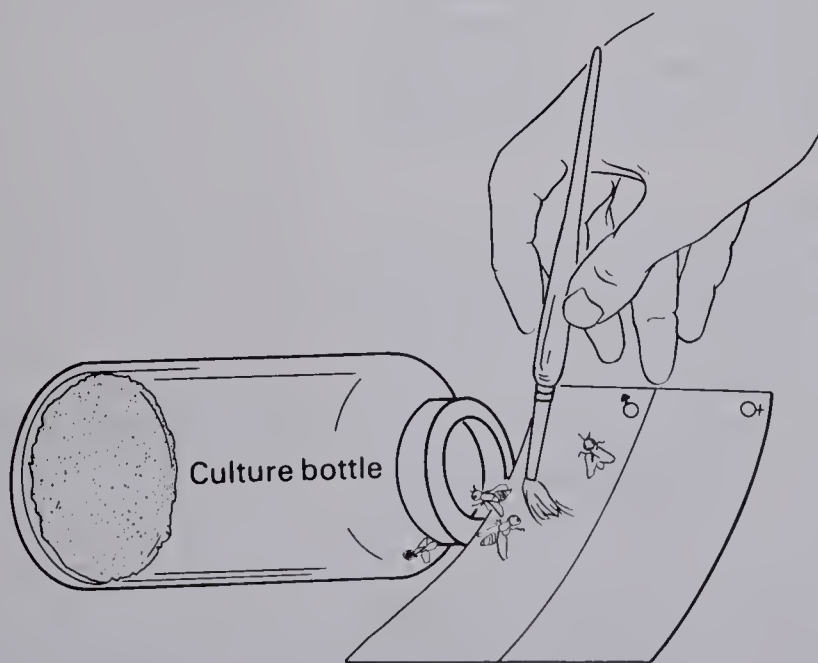
When the flies are anesthetized (not moving), shake them out onto the file card. Recork the etherizer. If the flies begin to move while you are examining them, you must anesthetize them again. Put one drop of ether on the cotton in the petri dish. Invert this reetherizer over the waking flies until they stop moving.

Use the brush to line up the flies on the center line. Examine the flies with a hand lens or dissecting microscope to determine their sex. The female's abdomen comes to a point at the posterior end. The male's abdomen is smaller, blunt, and has dark bars across the dorsal side. (The bars may not be present in young males.) On the male's ventral side there is a brown spot at the tip of the abdomen.

With the brush, push the male flies to the ♂ half of the card. Push the females to the ♀ half. All team members should verify the work.

1. How many males and how many females are there?

Gently brush *two females* and *two males* onto a second file card. Place the culture bottle on its side and brush the two males and two females into it. Insert the stopper. Leave the bottle on its side until the flies revive, so they will not get stuck in the medium.



Label the bottle with your name, period, the date, and a plus sign (+), the symbol used to designate wild-type flies. Set the bottle aside.

Brush the remaining flies on the other file card into the morgue. This will painlessly kill the flies.

Take Care: Do not anesthetize flies for longer than two minutes at a time.

Take Care: Do not let the flies escape. It is better to swat them than to let them fly away. These fruit flies are unlikely to upset the local environmental balance. However, they can cause problems in genetics experiments and become an annoyance.

Next, obtain a holding bottle of mutant-strain flies and another culture bottle. Follow the procedure you used with the wild-type flies. In addition to sexing the flies, determine the nature of the mutation.

2. How many males and how many females are there?

-
3. What is the nature of the mutation?
-

Transfer two males and two females to the culture bottle. Label with your name, period, date, and the type of mutation present in the culture. Dispose of the remaining flies in the morgue.

Place the bottles of wild-type flies and of mutant flies in a place designated by your teacher.

B. Crossing *Drosophila*

To have identifiable results when crossing strains, you must begin with a male and a virgin female. To get a virgin female, wait until the flies breed in the two bottles you have already prepared.

The females will lay eggs shortly after mating. After about eight days pupal cases will form on the sides of the jars. At that point, remove the adult flies and put them in the morgue. If you leave the adults in the jars, they will mate with the offspring, confusing the results of the cross.

When the new adults emerge, immediately remove them and determine their sex. Transfer *three females from one strain and three males from the other strain* to a clean culture bottle. You may decide which sex to take from which strain, but be certain that *all* the females come from one and *all* the males from the other.

Label the bottle "P-1 Cross" and indicate the strains and sexes (for example, "3 male mutants and 3 female wild-type," or vice-versa). Include your name, period, and the date on the label. Put the bottle in a place to incubate. Record the data under "P-1 Cross" on the *Drosophila* data sheet.

Dispose of the remaining flies in the morgue.

When the F-1 pupae appear, remove the parents and destroy them. When there are about 60 flies in the bottle, etherize them. Place three males and three females in a clean culture bottle. Label it "F-1 Cross" and include your name, period, and the date. Put the bottle in a place to incubate.

Examine a minimum of 50 flies from the F-1 generation to determine whether they have the phenotype of wild-type flies, mutant flies, or both. Record the data on the data sheet.

The offspring of the F-1 flies will be the F-2 generation. After the F-2 pupae appear, remove and dispose of the F-1 adult flies.

Examine at least 50 F-2 adult flies and note the phenotypes. Record your observations on the data sheet.

After you have finished observing the F-2 flies, dispose of them in the morgue.

Name _____ Date _____

Drosophila Data Sheet

P-1 Cross 3 $\frac{\text{sex}}{\text{sex}}$ *wild type* \times 3 $\frac{\text{sex}}{\text{sex}}$ $\frac{\text{nature of mutation}}{\text{nature of mutation}}$

	<i>P-1 Cross</i>	<i>F-1 Cross</i>
Date of Cross		
Date of Emergence		
Total Number of Flies Observed		
Number of Wild Type		
Number of Mutant		

ANALYSIS

4. What are the stages in the *Drosophila* life cycle? About how long does each pre-adult stage last?

5. Give two reasons why the parents are removed from the bottle when the pupae appear.

6. The six flies of the P-1 Cross were all pure bred for their traits. What is the genetic term for being purebred?

7. What Mendelian ratio of phenotypes would you expect in the F-1 generation in a cross where one parent is purebred for the dominant trait and the other parent is purebred for the recessive trait?

8. What ratio of wild-type to mutant flies actually occurred in the F-1 generation?
- _____
9. What Mendelian ratio would you expect in the phenotypes of the F-2 generation in a cross between members of the F-1 generation?
- _____
10. What ratio occurred in the F-2 generation?
- _____

FOLLOW-UP

The ratio of wild-type to mutant flies you observed may have differed from the expected ratio. You can find out whether your result is within a normal range of expected results, or is outside that range using the chi-square statistical test.

Chi-square Calculation

<i>Step</i>	<i>Wild-type</i>	<i>Mutant</i>	<i>Total</i>
1. Observed (O)			
2. Expected (E)			
3. Difference (D)			
4. D^2			
5. D^2/E			$\chi^2 =$

Fill in each line of the table as indicated by the following directions:

Line 1. Fill in the numbers of flies of each type you observed. Fill in the total number also. (Be sure to use actual numbers, not percentages.)

Line 2. Using the same total number as in Line 1, calculate the expected number of wild-type and mutant flies. (For example, if you observed 50 flies, write 50 in the Total column.) Put those numbers in the first two columns.

Line 3. Now subtract Line 2 from Line 1 in the first two columns.

Line 4. Square the difference between the expected and observed values for the wild-type and mutant categories.

Line 5. Divide each square by the expected number. Add the results for the first two columns and enter the sum as the chi-square value in the third column.

Next, the chi-square value must be compared with a table of probabilities. A simple table for two data categories (wild-type and mutant flies) is shown here. This table cannot be used in cases where there are more than two possible outcomes (more than two data categories).

Probability for 2 Data Categories

<i>Chi-square value</i>	<i>Probability that chance alone is responsible for the deviation (percent)</i>	
.0002	.99	Deviation not significant
.004	.95	
.064	.80	
.455	.50	
1.07	.30	
1.64	.20	
2.71	.10	Deviation significant
3.84	.05	
5.41	.02	
6.64	.01	
More than 6.64	Less than .01	

Probability refers to the likelihood your result was obtained by chance. If your chi-square value was .455, for example, the chance is .50 or 50 percent that you got that result by chance. The lower the probability, the less likely that your result was just chance variation in the expected ratio.

Notice that a chi-square value above 3.84, indicates a significant deviation. In research work, a result that has a low probability of being caused by chance is considered invalid. Usually scientists consider a probability of 5 percent or less as indicating that chance alone could not have resulted in the deviation of the outcome from the expected result.

What was the probability of getting the chi-square value you obtained? (Your value will probably not be exactly like any value on the table. Use proportional reasoning to estimate the probability.)

Was your result significantly different from the expected ratio? If so, what factor or factors may have caused the deviation?

51 Gene Expression and the Environment

PURPOSE

To investigate factors that cause chlorophyll development in tobacco leaves.

MATERIALS

100 tobacco seeds

aluminum foil

file card

2 petri dishes, 9 cm,
containing agar with carbon

wax pencil

INTRODUCTION

If you wish to make a chocolate cake, you need a recipe, the proper ingredients, and an oven. If any of these is unavailable, no cake can be made. Like bakers, cells need recipes, ingredients, and the proper environmental conditions to function. The cell's recipes are the genes, its ingredients are water and nutrients, and the proper environmental conditions include the pH level, temperature, and other factors.

The genes are the recipes for a cell's characteristics, or traits. Normally, a cell has two genes for each trait. The combination of genes a cell contains is known as its genotype.

The genes for a particular trait may be the same or they may be different. Alternate versions of genes for the same trait are known as alleles. Imagine having two different recipes for chocolate cake. They both contain the information for making chocolate cake, but the cakes are different. Similarly, different genes for the same trait produce different results. For example, the gene coding for blue and the gene coding for brown are different alleles for the trait eye color.

The expression of an organism's genes, or how the traits actually appear, is known as its phenotype. In the above example, blue eyes and brown eyes are both phenotypes. Gregor Mendel, whose work provided the foundation for the study of genetics, expressed the various phenotypes of a trait as a ratio. For example, if 75 percent of a generation has one phenotype and 25 percent has another, the ratio is 3:1.

Mendel found certain phenotypic ratios to be the most common: 3:1, 1:2:1, 1:1, and 9:3:3:1.

In this lab you will observe the trait leaf color in 100 tobacco plants of the same generation. There will be two phenotypes: green leaves and albino leaves, which appear yellow or colorless. The color of the green leaves is due to the development of chlorophyll.

PROCEDURE

You will grow tobacco seedlings in two petri dishes. The dishes contain agar to hold the seeds in place and as a source of water. The agar has been blackened with carbon to provide a dark background so that the colors of the seedlings can easily be seen.

Fold a file card in half and count out 50 tobacco seeds into the crease of the card. Using a pencil point, push the seeds one by one onto the surface of the agar. The 50 seeds should be distributed so that no two seeds are touching. Add 50 seeds to the second dish in the same manner.

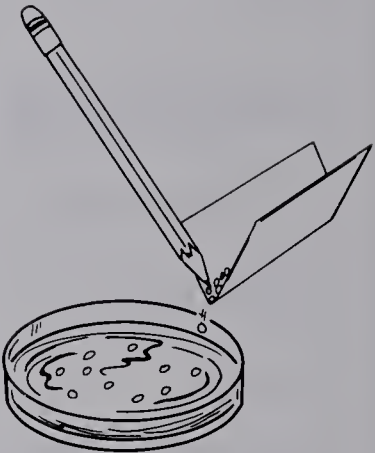
Place the lids on both petri dishes. Write your name and class period on both dishes with a wax pencil.

Wrap one of the dishes with aluminum foil so that all light is excluded. Place this dish in a drawer or cabinet. Place the unwrapped dish in a well-lighted area.

Most of the seeds should germinate within seven to ten days. Observe the lighted dish daily to watch for germination. *Do not* disturb the unlighted dish.

When the seedlings in the lighted dish are developed enough to count, remove the foil from the unlighted dish. Observe the seedlings in both dishes, noting how many are green and how many are albino (yellow or colorless).

Record your data on the data chart below and on the chart on the chalkboard. Transfer the class data from the chalkboard onto your chart. Using the class data, calculate the percentage of green and of albino plants in each dish and record the percentages on your chart.



percent green or albino plants =

$$\frac{\text{number of green or albino plants in dish}}{\text{total plants in dish}} \times 100$$

	Lighted Dish		Unlighted Dish	
	green	albino	green	albino
Your Data				
Class Data				
Percentage of Total Plants				

1. What environmental condition for the development of chlorophyll did you test?

2. What was the control in this experiment?

3. Do the plants in the unlighted dish contain chlorophyll? How can you tell? What caused this condition—the plants' genotype and/or the environmental conditions?

4. If you put the unlighted dish in the light, would the leaf color of any of the plants change? If so, to what color? About what percentage of the plants would change?

Place the unlighted dish in a well-lighted place for two days. At the end of that time, observe the seedlings.

5. How many seedlings are green and how many are albino? Do the results confirm your prediction?

6. According to these results, would your answer to question 3 now be the same or different? If different, how would you answer that question now?

ANALYSIS

7. What is the ratio of green to albino seedlings in each of the dishes (after both have been in the light)?

8. To which common Mendelian ratio (see the introduction) are your ratios most similar? Explain any difference between your ratios and the closest Mendelian ratio.
-
-
9. What is the most likely genotype of the albino plants grown in light? Use G to represent the green gene and g for the albino gene. Circle your answer.
- GG Gg gg
10. What is the genotype of the parent plants that could have produced all these tobacco seeds? Circle one set.
- GG × GG GG × Gg Gg × Gg gg × gg
11. Which one of the following statements is supported by the results of this lab? Circle the letter.
- a. The environment can mask the expression of genes.
 - b. An organism will have the traits dictated by its genes.
 - c. If the environmental conditions are correct, the organism will have a trait even though it lacks the genes for that trait.

FOLLOW-UP

Use the Chi-square test to compare your data with the expected Mendelian ratio. See the Probability lab for instructions on using this statistical method of evaluating data.

52 Human Blood Types

PURPOSE

To learn what blood types are and how to type blood.

MATERIALS

fresh anti-A serum	slides
fresh anti-B serum	eyedropper
absorbent cotton swabs	sterile disposable lancets
ethyl alcohol	toothpicks
compound microscope	wax pencil

INTRODUCTION

Human blood is classified according to the presence or absence of proteins called antigens on the surface of red blood cells. In discussions of blood types, antigens are commonly referred to as agglutinogens.

Blood may be categorized into four types: type A, type B, type AB, and type O. The blood type gets its name from the kinds of agglutinogens present. Type A has agglutinin A, type B has agglutinin B, type AB has both agglutinogens, and type O has neither agglutinin.

The blood plasma contains proteins called antibodies—or, more commonly, agglutinins—that react with certain agglutinogens. This reaction causes red blood cells to clump together, or agglutinate.

Each kind of agglutinin reacts only with a specific agglutinin. For example, if agglutinin A is mixed with anti-A agglutinin, clumping will occur. As you might guess, your own blood plasma does not contain the agglutinin that would cause your red blood cells to clump. But it does contain agglutinins that would react with “foreign” agglutinogens. For example, a person with type A blood has red cells that contain agglutinin A and plasma that contains anti-B agglutinin. Type A blood has anti-B agglutinin, type B has anti-A agglutinin, type AB has neither agglutinin, and type O has both agglutinins.

In this lab you will learn how knowledge of agglutinogens and agglutinins is important in making blood transfusions, how heredity determines blood types, and how to type your own blood.

PROCEDURE

A. Blood Transfusions

Before people knew about the presence of agglutinogens and agglutinins in the blood, blood transfusions were often fatal. When the wrong type of blood was transfused, the red blood cells clumped. Clumped cells in blood vessels could clog capillaries, damage vital organs, and cause death. Only after the discovery of blood groups in the early 1900s did transfusions become relatively safe.

In transfusions, the main concern is about the clumping of the donor's red blood cells. For example, if type A blood is given to a type B recipient, the recipient's agglutinins will cause the donor blood to clump. The agglutinins in the donor blood will also react with the recipient's agglutinogens, but it causes only a small amount of clumping and is usually not dangerous. This is because there are so few donor agglutinins in relation to the great volume of the recipient's blood. So, in general, it is clumping of the donor's—not the recipient's—blood that can cause harm.

To prevent clumping, the medical staff determines the blood types of the donor and recipient before doing a transfusion. For example, a type A recipient can safely receive a transfusion of either type A or type O blood. The type A recipient should not receive either type B or type AB blood because the recipient's anti-B agglutinin would cause clumping of type B and type AB red blood cells in the donor blood.

Complete the following chart. Remember, be concerned only about the effect of the recipient's agglutinins on the donor's agglutinogens.

<i>Blood Type of Recipient</i>	<i>Blood Types of Possible Donors</i>
A	
B	
AB	
O	

1. According to the chart, what blood type does a universal donor (a person with blood that can be transfused into all types) have? Why would this type not be clumped by other blood types?

2. What blood type does a universal recipient (a person who can receive all types of blood) have? Why would this type not cause clumping of other types?

B. Genotypes of Blood Groups

Blood types are determined by the genes. Genes for a trait usually occur in only two alternate versions called alleles, one of which is dominant and one of which is recessive. Some traits, however, have multiple alleles. There are three alleles for blood type:

A = allele producing agglutinin A

B = allele producing agglutinin B

O = allele producing no agglutinogens

The A and B alleles are dominant and the O allele is recessive. When the A and the B alleles occur together in a genotype they are codominant and produce both A and B agglutinogens.

3. On the following chart, write all the possible genotypes for each blood group. The possible genotypes are: AA, AO, BB, BO, AB, and OO.

<i>Phenotype</i>	<i>Agglutinin</i>	<i>Agglutinin</i>	<i>Genotype</i>
type A blood	A	anti-B	
type B blood	B	anti-A	
type AB blood	A, B	none	
type O blood	none	anti-A, anti-B	

C. Typing Your Blood

You can determine your blood type by performing a simple lab test.

With a wax pencil, draw a line across the middle of a clean slide, dividing it in half. Label the halves A and B.

Wash your hands with soap and water, then dry them. Dip a cotton swab in alcohol and use it to clean the third fingertip of your left hand. With your left thumb, stroke this finger in an upward direction three or four times. This forces the blood to your fingertip. On the final stroke, hold your thumb near the fingertip and press down. With an unused sterile lancet, puncture your fingertip.

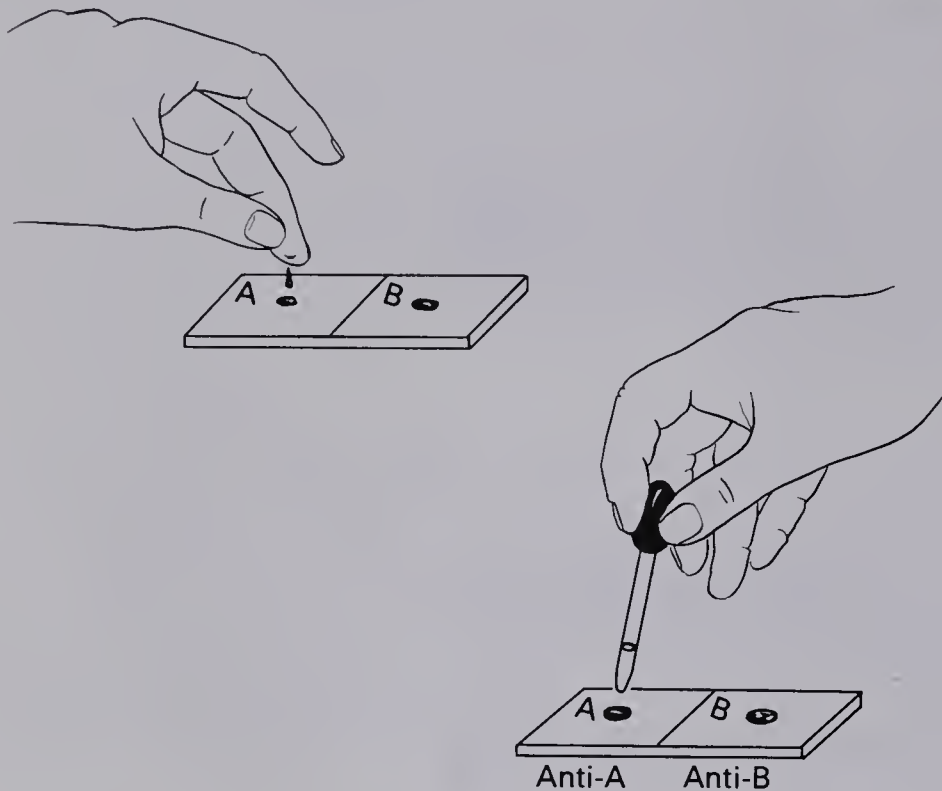
Caution: Do not use a lancet that someone else has already used. The lancet must be sterile.

Put one drop of blood in box *A* on your slide and one drop in box *B*. Immediately dispose of your lancet so that no one else will use it.

Place one drop of anti-A serum (colored blue) on the blood in box *A*. Mix the blood and serum with a clean toothpick. Dispose of the toothpick immediately after use.

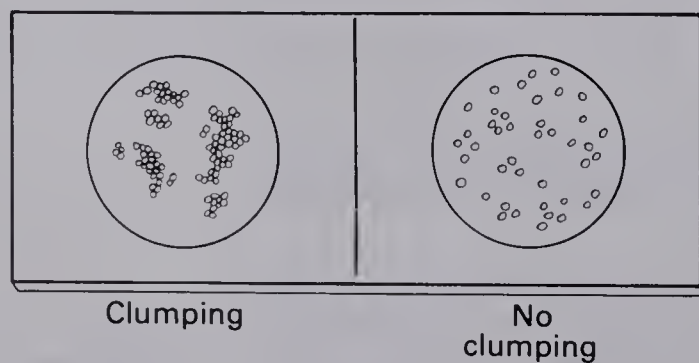
Place a drop of anti-B serum in the blood in box *B*. Mix with a clean toothpick and then dispose of the toothpick.

Caution: Do not let the dropper touch the blood, or get serum on your fingers.



For three or four minutes, slowly tilt the slide back and forth to keep the solutions in motion. Be careful not to let the liquids in box *A* and in box *B* mix. Watch for clumping.

Observe the slide under low power with a compound microscope. (If you wish to use high power, add a coverslip to the slide.) Clumped blood cells in box *A* indicate the presence of agglutinin A. Clumping in box *B* indicates the presence of agglutinin B. If there is no clumping in either box, neither agglutinin is present.



4. What is your blood type as indicated on your slide?

5. Collect blood type data from the entire class and calculate the percentages of each type in the class. Enter your data on the following chart.

	<i>Number of People with Blood Type</i>	<i>Percentage of Class with Blood Type</i>
A		
B		
AB		
O		

ANALYSIS

The approximate frequencies of blood types found in the population of the United States are as follows:

Type	Frequency
O	46%
A	40%
B	10%
AB	4%

6. Are the United States data fairly close to the class data (within 5-10 percent)? If you determined blood types for the whole school, would the school data be closer than the class data to the United States data? Explain your answer.

7. If you were married to a person with type A blood, what blood types could your children have?

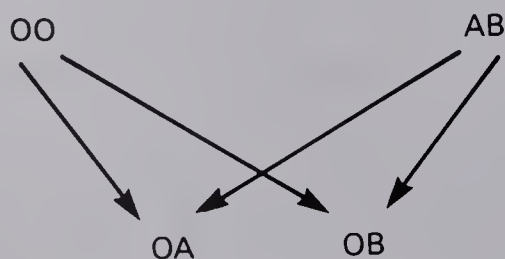
<i>Student</i>	<i>Children</i>

8. If you were married to a person with type O blood, what blood types could your children have?

<i>Student</i>	<i>Children</i>

Mr. and Mrs. Mott have type A blood. Mrs. Mott gives birth to a baby delivered by bumbling old Dr. Elliott. Dr. Elliott mixes up the Mott baby with two others. Baby 1 has type B blood. Baby 2 has type O blood. Baby 3 has type AB blood.

9. Which baby belongs to the Motts? Show the possible genotypes of the parents and the offspring. (See the illustration as an example.)



10. Mr. and Mrs. Billings have type A and type B blood, respectively. Baby X has type A blood. Baby Y has type B blood. Baby Z has type O blood. Which baby could be theirs? Show the possible genotypes of the parents and offspring.

11. Which statement is more nearly correct? Explain your choice.
- By finding the blood types of parents and a baby, it is possible to determine that the baby is their offspring.
 - By finding the blood types of parents and a baby, it is possible to determine that the baby is not their offspring.

FOLLOW-UP

Blood is also typed by systems other than ABO typing. Another inherited blood type is the Rh factor. Rh agglutinogens were first found in rhesus monkeys; hence, the name Rh.

People who have the Rh agglutinin have Rh-positive blood. Those who do not have the Rh agglutinin in their blood are Rh-negative. In the United States 85 percent of the people have Rh-positive blood.

The blood plasma of Rh-negative people does not contain the agglutinin against the Rh agglutinin. If Rh-positive blood is transfused into an Rh-negative person, the recipient's blood will begin making anti-Rh agglutinins. A second transfusion of Rh positive blood will cause agglutination, which may be fatal.

Rh is controlled by Rh genes, not by ABO genes. The gene for Rh-positive blood is dominant over the gene for Rh-negative blood.

If your class has Rh-typing equipment available, find out whether you are Rh-positive or Rh-negative. Follow your teacher's instructions.

53 Population Dynamics

PURPOSE

To determine the frequency of genes in a population through the use of the Hardy-Weinberg Law.

MATERIALS

paper

pencil

INTRODUCTION

In Hoot Woods there are two kinds of mice: those with colored fur and those with albino (white) fur. These phenotypes have been recorded over several generations in a pedigree. The pedigree shows that two albino mice can have only albino offspring, and that two colored mice or one colored mouse and one albino mouse can have both colored and albino offspring. Therefore, the gene for colored fur is dominant and the gene for albino fur is recessive.

The number of a particular gene in a population is known as the gene frequency of that gene. Frequency is expressed as either a percent or a decimal. For example, in an entire population of mice 80 percent of the genes for fur color might be for albino fur and 20 percent for colored fur. The gene frequency of the albino gene is therefore 80 percent, or 0.8.

In stable environmental conditions, the frequency of genes for a trait in a genetically balanced population that mates randomly tends to remain the same. Thus, if the environment does not change, the frequency of the genes for albino fur will remain 0.8 or 80 percent, generation after generation. The maintenance of gene frequencies in this way is known as the Hardy-Weinberg Law. In this lab you will learn the mathematical expression of this law and how it applies to actual populations.

"I didn't count on you showing up!"



PROCEDURE

Let *A* stand for the gene for colored fur, and *a* for the gene for albino fur. Mice with the *AA* or the *Aa* genotype have colored fur. Mice with the *aa* genotype have albino fur. Here are the genotypes of 200 mice:

aa	aa	AA	Aa	aa	AA	aa	Aa	aa	Aa	aa	aa	AA	aa	aa
AA	aa	aa	Aa	aa	Aa	aa	aa	aa	Aa	aa	Aa	aa	aa	Aa
Aa	AA	aa	aa	Aa	aa	Aa	aa	aa	Aa	aa	aa	Aa	aa	Aa
aa	Aa	aa	Aa	Aa	Aa	Aa	aa	AA	Aa	Aa	aa	Aa	aa	aa
aa	aa	Aa	aa	aa	Aa	aa	Aa	aa	aa	Aa	aa	aa	Aa	AA
aa	aa	aa	Aa	Aa	aa	AA	aa	Aa	aa	aa	Aa	aa	aa	Aa
Aa	aa	Aa	AA	aa	Aa	aa	Aa	aa	Aa	aa	Aa	aa	aa	Aa
aa	Aa	aa	aa	Aa	aa	Aa	aa	aa	aa	AA	aa	Aa	Aa	Aa
Aa	aa	AA	Aa	aa	Aa	aa	Aa	aa	Aa	aa	Aa	aa	Aa	Aa
Aa	Aa	aa	Aa	AA	Aa	Aa	aa	Aa	aa	Aa	aa	Aa	aa	Aa
aa	Aa	Aa	aa	Aa	aa	Aa	AA	aa	Aa	aa	Aa	aa	Aa	aa
Aa	Aa	Aa	aa	aa	Aa	Aa	aa	Aa	aa	AA	Aa	aa	Aa	aa
aa	Aa	aa	Aa	Aa	aa	Aa	Aa	aa	aa	aa	aa	aa	AA	aa
AA	Aa	Aa	AA	AA										

To determine the frequency of these genes in the population, count the total number of genes, the number of *As*, and the number of *as*. Use the following formulas to calculate the gene frequencies. When calculating frequencies, use the decimal notation rather than the percent.

frequency of *a* = $\frac{\text{number of } a \text{ genes}}{\text{total number of genes } (A + a)}$

frequency of *A* = $\frac{\text{number of } A \text{ genes}}{\text{total number of genes } (A + a)}$

1. Calculate the frequency of the genes in the population. Express your answer as a decimal.

Frequency of the a gene = _____

Frequency of the A gene = _____

If you have done the calculations properly, the sum of the two frequencies will be 1.0, or 100 percent.

In the Hardy-Weinberg equations, the frequency of the dominant gene in a population is designated p , and the frequency of the recessive gene is designated q . Thus, the general equation for the frequency of genes in a population is:

$$p + q = 1.0 \text{ (or 100 percent)}$$

Now, examine the relationship between the frequency of albino mice and the frequency of the albino gene in the population. Use the following equation to determine the frequency of albino mice.

$$\text{frequency of albino mice} = \frac{\text{number of albino mice (aa)}}{\text{total number of mice}}$$

2. Calculate the frequency of albino mice in the population.
3. Is this frequency greater or smaller than the frequency of the a gene?

4. If q = the frequency of a (the recessive gene) in the population, is the frequency of albino mice in the population equal to $2q$ or to q^2 ?

You have learned that $p + q = 1$, and that q^2 = the frequency of the recessive phenotype in the population. With this information, you can

determine the frequency of the genes in a population, even if you do not know all the genotypes (as you did with the mice).

Consider the following situation. There are 100 owls in Hoot Woods. You can make some easy calculations to determine the frequencies of the phenotypes and the frequencies of the genes in this population. Of the 100 owls, 64 have the dominant phenotype of a long tail, and 36 have the recessive short tail. With S as dominant and s as recessive, the genotype must be: SS or Ss for long tail, and ss for short tail.

5. What is q^2 (the frequency of the recessive trait, ss)?

6. What is the frequency of the dominant trait?

7. If $q^2 = .36$, what does q equal?

When you know the value of q , you can calculate p from the equation, $p + q = 1$. By subtracting q from both sides of the equation, you get $p = 1 - q$.

8. Calculate p for the owl population.

If q^2 is the frequency of the homozygous recessive trait (ss) in the population, p^2 must be the frequency of the homozygous dominant traits (SS).

9. Calculate p^2 (the frequency of the homozygous dominant trait, SS).

The only frequency that you have not determined is the frequency of the heterozygotes in the population, designated $2pq$. You know the first two addends in the following equation:

	frequency of homozygous recessive owls (q^2)
+	frequency of homozygous dominant owls (p^2)
+	frequency of the heterozygotes ($2pq$)
<hr/>	
	1.0 or 100 percent

10. Calculate $2pq$ (the frequency of the heterozygous trait, Ss).

The two Hardy-Weinberg equations are:

$$p + q = 1.0$$

and

$$p^2 + 2pq + q^2 = 1.0$$

The Hardy-Weinberg Law indicates that the frequency of the genes for a trait will remain constant in a genetically balanced population living in a stable environment. Therefore, a recessive or a dominant gene can have a very low frequency in a population and still remain in the population generation after generation. It is for this reason that recessive traits do not simply disappear from a population over time, which one might initially expect.

ANALYSIS

11. In a population of 200 mice, 8 have short tails, which is a recessive trait. The rest have long tails. Determine the frequencies of the genotypes and of the dominant and recessive genes.

$$\begin{array}{lll} q^2 = \underline{\hspace{2cm}} & q = \underline{\hspace{2cm}} & p = \underline{\hspace{2cm}} \\ p^2 = \underline{\hspace{2cm}} & 2pq = \underline{\hspace{2cm}} & \end{array}$$

The frequency with which mice of any two genotypes mate can be calculated as follows. (We are assuming that tail length does not influence selection of a mate.) The frequency of the males with a certain genotype (such as homozygous dominant, p^2) is multiplied by the frequency of the females with a certain genotype (such as homozygous recessive, q^2), or $p^2 \times q^2$.

12. Using the information obtained in question 11, what is the frequency of the mating between homozygous dominant males and homozygous recessive females?
- _____

13. The cross $2pq \times 2pq$ describes the mating of what two kinds of individuals?

14. Match each of the following symbols with the phrase that defines it.

- | | | |
|-------|-------|---|
| p | _____ | a. The frequency of homozygous dominant individuals in the population. |
| q | _____ | b. The frequency of the dominant gene. |
| p^2 | _____ | c. The frequency of heterozygotes in the population. |
| $2pq$ | _____ | d. The frequency of the recessive gene. |
| q^2 | _____ | e. The frequency of homozygous recessive individuals in the population. |

15. Write the two Hardy-Weinberg equations.

54 Pasteur's Experiment

PURPOSE

To confirm Louis Pasteur's evidence disproving spontaneous generation.

MATERIALS

Per team of students:

400 mL nonsterile beef broth	5 flasks, 250-mL
aluminum foil	nonabsorbent cotton
autoclave	S-shaped glass tubing

INTRODUCTION

For many centuries it was believed that spontaneous generation—living things arising from nonliving things—could occur. In old books one can read of leaves falling into water and becoming fish, of a recipe for generating mice from grains of wheat and a dirty shirt, and many other tales that now seem amusing.

One belief was that wormlike maggots arose spontaneously from decaying meat. But Francesco Redi, an Italian doctor, suggested that maggots were the larval stage of flies and that they hatched from eggs deposited on decaying meat. In 1668 he designed an experiment to test the hypothesis that maggots were produced spontaneously.

Redi put meat in open flasks, sealed flasks, and screen-covered flasks. Maggots developed in the open flasks only. However, on the screens of some of the flasks, flies laid eggs, which hatched into maggots.

Redi's experiment presented the first convincing evidence against spontaneous generation. Almost two hundred years later, French chemist Louis Pasteur set out to fully disprove the theory.

Many people believed that spontaneous generation produced the bacteria found in broth exposed to the air. The bacteria supposedly came from the broth in the presence of an "active principle." The active principle could not work without air.

Pasteur, however, hypothesized that the bacteria were carried to the broth by dust particles in the air. In 1862 he devised an experiment that proved his hypothesis and led to the abandonment of the idea of spontaneous generation.

In this lab you will recreate Pasteur's famous experiment.

PROCEDURE

For his experiment, Pasteur sterilized some of the broth by boiling it for several hours. Some of the broth for your experiment will be sterilized in an autoclave. An autoclave is a type of pressure cooker that heats liquids to temperatures higher than their normal boiling points. These high temperatures usually kill all living things in the liquids.

Each team of students should prepare five flasks as follows. Label the flasks *A*, *B*, *C*, *D*, and *E*. Write a team name on each of the flasks.

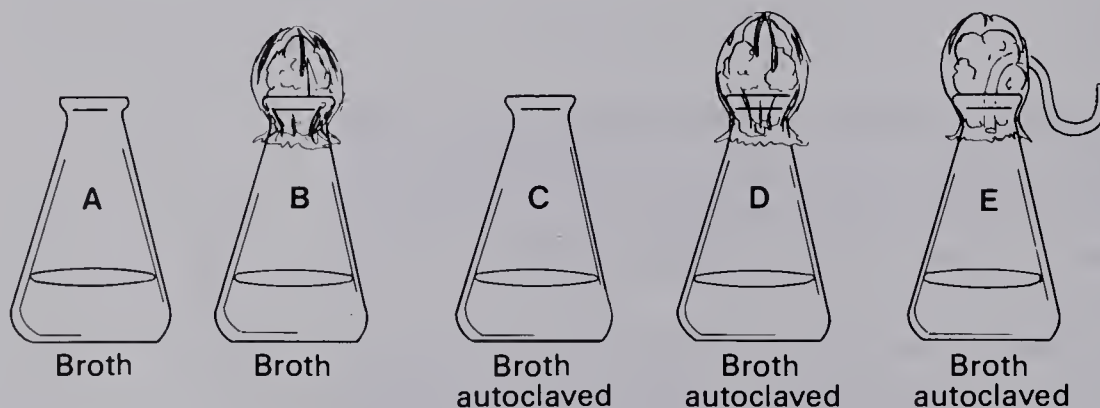
Add 75 mL of unsterilized broth to each flask; they should be approximately one third full. Leave flasks *A* and *C* uncovered.

Insert a wad of nonabsorbent cotton into the neck of flask *B* to serve as a stopper. Seal it with aluminum foil and crimp the foil around the lip of the flask.

Insert nonabsorbent cotton into flask *D* and cover it loosely with aluminum foil. Do not crimp the foil.

Insert cotton into flask *E* and cover it loosely with foil. Carefully insert an S-shaped glass tube through the foil and cotton. The bottom of the tube must be above the surface of the broth.

Give flasks *C*, *D*, and *E* to your teacher to be autoclaved. After the autoclaving, crimp the foil around the lips of flasks *D* and *E*.



Store all five flasks in a place where they will not receive any direct sunlight, as designated by your teacher.

Growth of bacteria will cause the clear broth to become cloudy. Predict which flasks will become cloudy, showing growth, and which will remain clear, showing no growth. Write “growth” or “no growth” for each flask in the “Prediction” column of the chart.

Observe the flasks every day for 10 days.

<i>Flask</i>	<i>Prediction</i>	<i>Observation</i>
A		
B		
C		
D		
E		

ANALYSIS

1. Which flasks are controls for the experiment?

2. What conclusion would you reach if no growth occurred in flasks *C*, *D*, and *E*, and growth occurred in *A* and *B*?

3. Did the S-shaped tube in flask *E* allow anything to enter the flask? What? Did the tube trap anything, preventing it from entering the flask? What? (Examine the tube closely for evidence.)

4. No growth in flask *E* would be convincing evidence against spontaneous generation, and no growth in flask *D* would not disprove the theory. Why?

5. Where did the bacteria come from that appeared in flask *C*?

6. What are two applications in everyday life for the knowledge gained from Pasteur's experiment?

7. Redi's experiment seemed to conclusively disprove spontaneous generation. Then, some years later, the invention of the microscope allowed scientists to see microorganisms. The existence of those tiny organisms revived the idea of spontaneous generation. Why might it have revived the theory?

FOLLOW-UP

With guidance from your instructor, prepare and stain a slide of the bacteria from a cloudy flask. Examine the slides under high power with a compound microscope.

55 Variation Within Species

PURPOSE

To observe variation in three species.

MATERIALS

Per team of 2:

10 preserved adult grasshoppers

25 soaked bean seeds

centimetre ruler

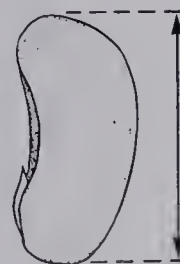
INTRODUCTION

Every living organism interacts with its environment. These interactions are affected by the organism's characteristics, which are largely determined by its heredity. Interactions between heredity and environment may result, over time, in a great deal of variety within a species.

When Charles Darwin visited the Galapagos Islands, he found 13 species of finches. The main difference among the species was the shape of the beaks. All 13 species are thought to have a common ancestor. In the original species of finch, beaks probably varied somewhat from bird to bird. Some beaks were a little longer, some were more curved, and so on. Certain of these variations proved beneficial for gathering the different kinds of food available on the island. One beak was well-adapted to getting insects, another to seeds, another to cactus.

When two birds with the same variation of beak mated, their offspring probably had the same type of beak. If the variation was advantageous to food-gathering, the offspring were well-adapted to survive and reproduce in their environment. In this way, favorable variations were passed on over time. Unfavorable variations disappeared, since birds with the unfavorable variations did not survive.

As with the finches, variation within every species plays an important part in its survival and evolution. In this lab you will examine variations in bean seeds, human index fingers, and grasshopper femurs.



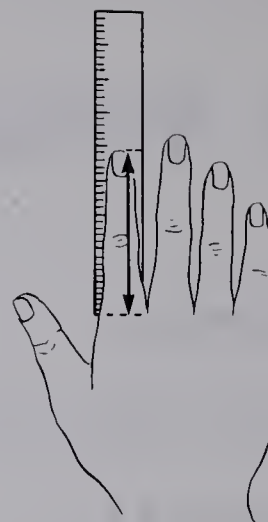
PROCEDURE

A. Bean Seeds

Remove the seed coats from 25 soaked bean seeds. Separate the cotyledons of each seed and dispose of one of them. Measure the other

cotyledon of each seed as illustrated. Average your data and write the number on the chalkboard under *Average Seed Length*.

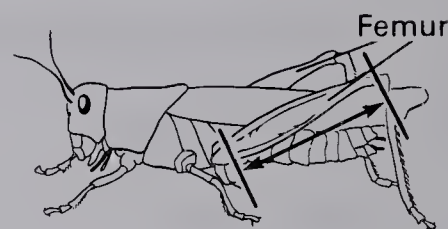
1. What is the average for the class data?



B. Index Fingers

You will measure the index finger of your right hand. Place a ruler on the desk. Place your hand on the ruler so that you can measure from the notch between the index finger and the middle finger to the tip of the index finger. Do not include your fingernail in the measurement. Record your data on the chalkboard under *Average Finger Length*.

2. What is the average for the class data?



C. Grasshopper Femurs

The femur, the leg bone above the knee is the longest bone in the body. In grasshoppers, femur are located on the jumping legs.

Measure one of the femurs on ten grasshoppers. When you finish, put the grasshoppers in the place designated by your teacher.

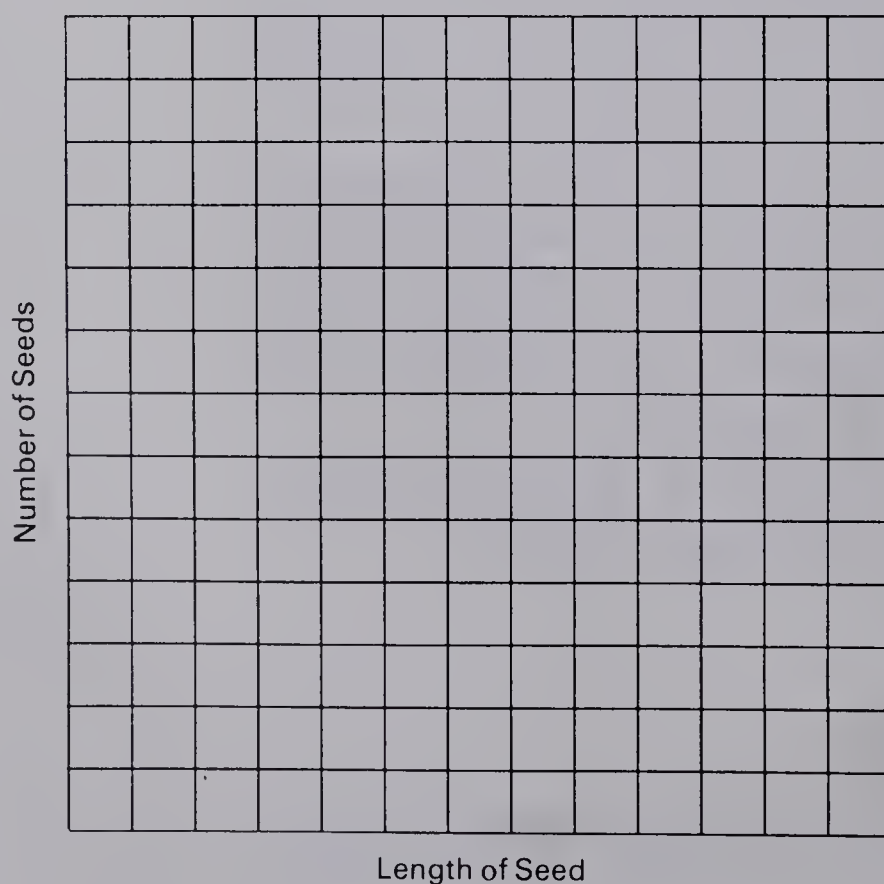
Average your data and record it on the chalkboard under *Average Femur Length*.

Take Care: Handle the grasshoppers carefully, so that they can be used by other classes.

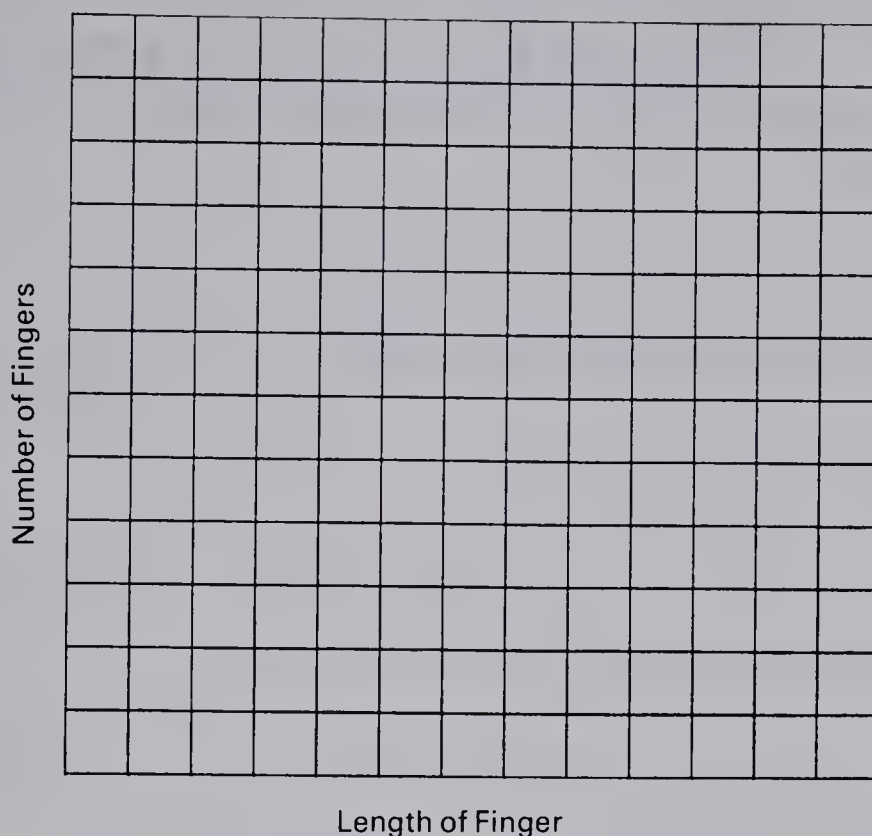
3. What is the average for the class data?

4. Prepare histograms of the class data. See the Graphing lab if you need help.

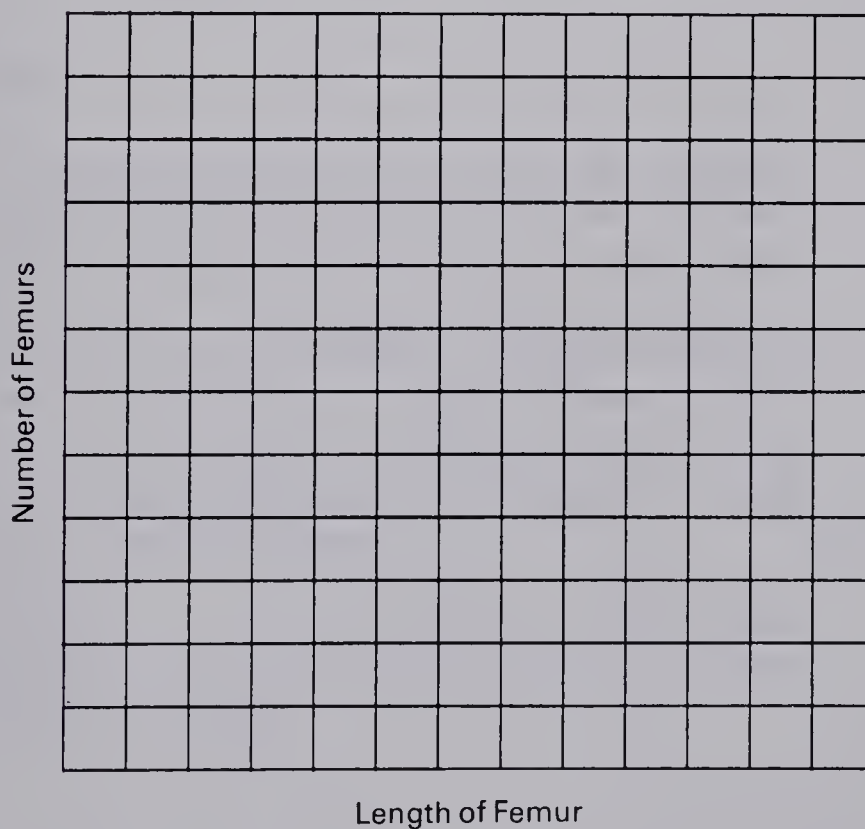
Seed Lengths



Index Finger Lengths



Grasshopper Femur Lengths



ANALYSIS

5. According to the histograms, which characteristic shows the greatest amount of variation? Which shows the least amount of variation?

6. Circle the word in parentheses that makes the following statement correct:

According to the histogram of bean seed lengths, as the degree of variation from the average increases, the frequency of that variation (increases, decreases).

7. Does the above statement also apply to the histograms of finger lengths and femur lengths?

8. List and explain the selective advantages of large beans.

9. List and explain the selective advantages of small beans.

10. List and explain the selective disadvantages of short femurs in grasshoppers.

11. Is finger length in humans related to the ability to survive?

12. Think of a situation in which long fingers in humans would be advantageous for survival.

56 Variation in Bacterial Resistance to Antibiotics

PURPOSE

To study variation within a species by determining whether all bacteria in a pure culture are identical.

MATERIALS

broth culture of <i>Bacillus subtilis</i>	Bunsen burner
3 tubes of nutrient agar	pencil
1 tube of streptomycin agar	test tube for culture
inoculating loop	wax pencil
2 petri dishes	

INTRODUCTION

In a pure strain of any organism, it may appear that all the individuals are the same. For example, if all the individuals in a strain of bacteria are the same, they should all react in the same way to an antibacterial agent such as an antibiotic.

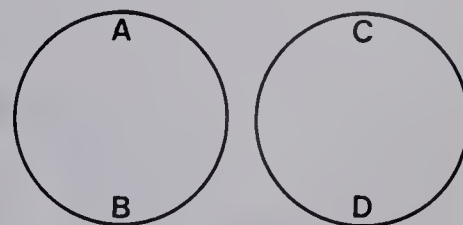
However, they might actually act differently. If some of the cells have mutated, they may have changed into slightly different kinds of cells. If so, these bacteria might be able to withstand higher concentrations of antibiotics than other cells. In other words, there would be variation in the ability to resist antibiotics.

In this lab you will test the effects of different concentrations of the antibiotic streptomycin on a culture of *Bacillus subtilis*. Like other species of bacteria, this common bacterium reproduces by binary fission. The rate of reproduction is high.

PROCEDURE

Obtain two covered petri dishes; set the covers aside. On the outside of the bottom of one dish, write your name and the letters *A* and *B* with a wax pencil (as shown in the drawing). Label the bottom of the other dish with your name and the letters *C* and *D*.

You are going to pour agar into each dish. Agar solidifies at 42°C. Pour quickly—if you allow the temperature of a tube of liquid agar to drop below 42°C, the agar will harden. You must then reheat it to 100°C to liquefy it again.



Take Care: Do not let the agar media in the tubes cool before you pour them.

Remove the stopper from one tube of liquid streptomycin agar. Pour the liquid into dish A-B. Cover the dish immediately.

Place a pencil under the A on the petri dish so that the streptomycin agar is distributed as shown in the drawing.

When the agar is solidified, remove the pencil and place the dish flat on the work area. Then pour a tube of liquid nutrient agar on top of the solidified agar in dish A-B. After the nutrient agar has solidified, cover the dish. The result should look like the drawing.

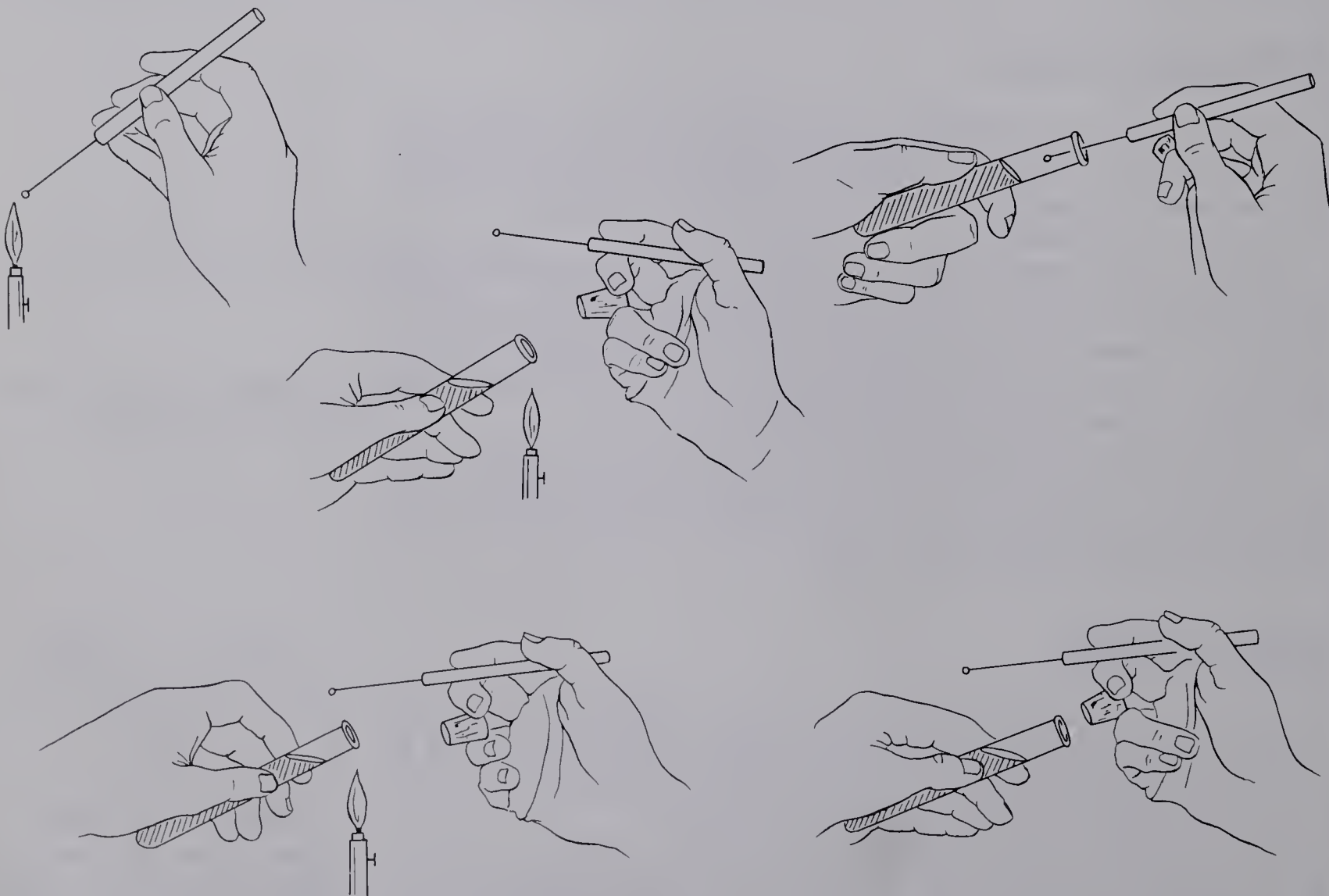
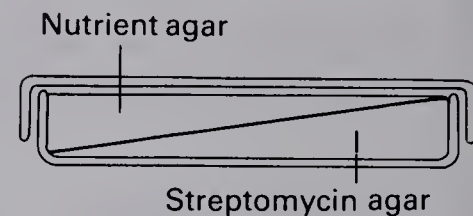
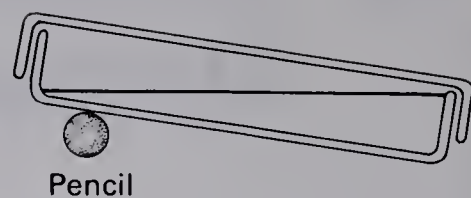
The nutrient agar contains all the nutrients necessary for the bacteria to grow. By preparing the A-B plate as you have, different concentrations of the antibiotic are found in different parts of the dish.

Obtain two more tubes of liquid nutrient agar. Pour them in the second petri dish (on a flat surface) and allow the agar to solidify. Cover the dish.

Once the agar in both petri dishes has cooled, obtain a broth culture of *Bacillus subtilis* and an inoculating loop. Heat the loop in a flame until it turns bright reddish-yellow; then allow it to cool. This process sterilizes the loop.

Remove the stopper from the broth culture and pass the lip of the container through a flame. This warms the opening so that air will tend to move out of the container, not into it. The room air may contain spores, which would contaminate the culture.

Dip the cool sterile loop into the *Bacillus subtilis* culture. Remove the loop, flame the lip of the container again, and replace the stopper.

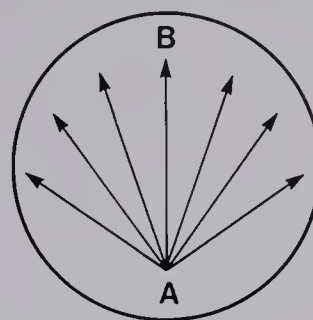


Carefully raise the lid of the petri dish containing the streptomycin agar. Streak the agar in the manner shown in the illustration from *A* to *B* first, then work your way to the sides. Do not break the surface of the agar.

When you have finished the *A-B* plate, sterilize the inoculating loop and repeat the process on the *C-D* plate. Start at *C* and go to *D*.

When you are finished, sterilize the loop to remove any bacteria and put the loop away.

Cover the petri dishes, invert them, and place them in an incubator or dark drawer. The agar will be hanging from the top of each dish.



1. Where in the *A-B* plate is the concentration of antibiotic the highest?

2. Where in the *A-B* plate is the concentration of antibiotic the lowest?

3. If the bacteria do not grow at all in either plate, how would you interpret this?

4. If bacteria do not grow in plate *A-B*, but do grow in plate *C-D*, how would you interpret this?

5. If all the bacteria are affected uniformly, how would you expect plate *A-B* to look?

6. If some of the bacteria are affected differently, how would you expect plate *A-B* to look?

After 24 hours, remove the petri dishes from the incubator or dark drawer and observe them.

7. Draw a sketch of the appearance of both plates.

ANALYSIS

8. What is the purpose of plate *A-B*?

9. What is the purpose of plate *C-D*?

10. Describe the growth of bacteria in plate *A-B* and plate *C-D*.

11. Interpret the results you obtained. How do your results compare with your predictions?

57 A Predator-Prey Simulation

PURPOSE

To study the relationship between the sizes of predator and prey populations.

MATERIALS

Per team of 2:

cellophane tape

metre stick

masking tape

scissors

INTRODUCTION

Animals spend much of their time looking for and consuming food. Some eat plants, some eat meat, and some eat both. Many meat-eating animals obtain their meat by hunting other animals. The hunters are known as predators and the hunted animals are known as prey.

In this lab you will do a simulation of a predator/prey relationship, with owls as predators and mice as prey. In nature, owls and mice are often found living in forests. The forest in your simulation will be Hoot Woods.

Owls are excellent hunters. The various kinds of owls eat many different kinds of animals, including rabbits, squirrels, rats, mice, shrews, birds, fish, and insects. To simplify the simulation, you will limit the owls' food supply to mice.

PROCEDURE

Each team should cut out the 400 mice squares and 1 owl rectangle. Fold the rectangle in thirds to make an owl square about 6 cm on a side. Tape the three open sides using cellophane tape. The teams can prepare the squares at home prior to class, or at the beginning of this lab period.

Using masking tape, each team should mark off a square approximately 50 cm on a side. Make the square on a flat surface such as a lab table, a desk, or the floor. This square represents Hoot Woods, where the mice and owls live.

You will simulate 25 generations of owls and mice. The mice can be eaten and the owls can starve. Surviving mice and owls can reproduce.

To make calculations easier, each surviving mouse and owl will be considered capable of producing one offspring.

In each generation, the surviving mouse population will double to form the next generation. For example, if six mice are living in the woods and two are caught by an owl, then four mice will survive. These four mice will each produce one offspring, and the next generation will begin with eight mice. Remember, the number of offspring is always the same as the number of surviving mice. At any one time, the maximum mouse capacity of Hoot Woods is 400 mice.

In order to survive, each owl must catch at least three mice in every generation. If an owl does not catch three mice, it will starve. For each three mice that an owl catches, it produces one offspring. For example, if an owl catches eight mice it will reproduce two new owls, making a total of three owls to begin the next generation.

At the beginning of *each* generation there must be at least three mice and one owl in the woods. If the populations drop below these numbers (by being eaten or starving), new mice and owls will migrate in. For example, if just one mouse survives the first generation, just one offspring will be produced, for a total of two mice. One mouse must migrate in to bring the mice total to three. If all owls die, one owl must migrate in.

A. Hoot Woods Simulation

The simulation is played as follows. Place the mouse squares at random in Hoot Woods. Then, from a height of about 30 cm, drop the owl square into the woods. Try to hit as many mice as you can in one drop. When an owl square fully or partly covers a mouse square(s), that is a "catch." If there is more than one owl in a generation, drop the owl square once for each owl.

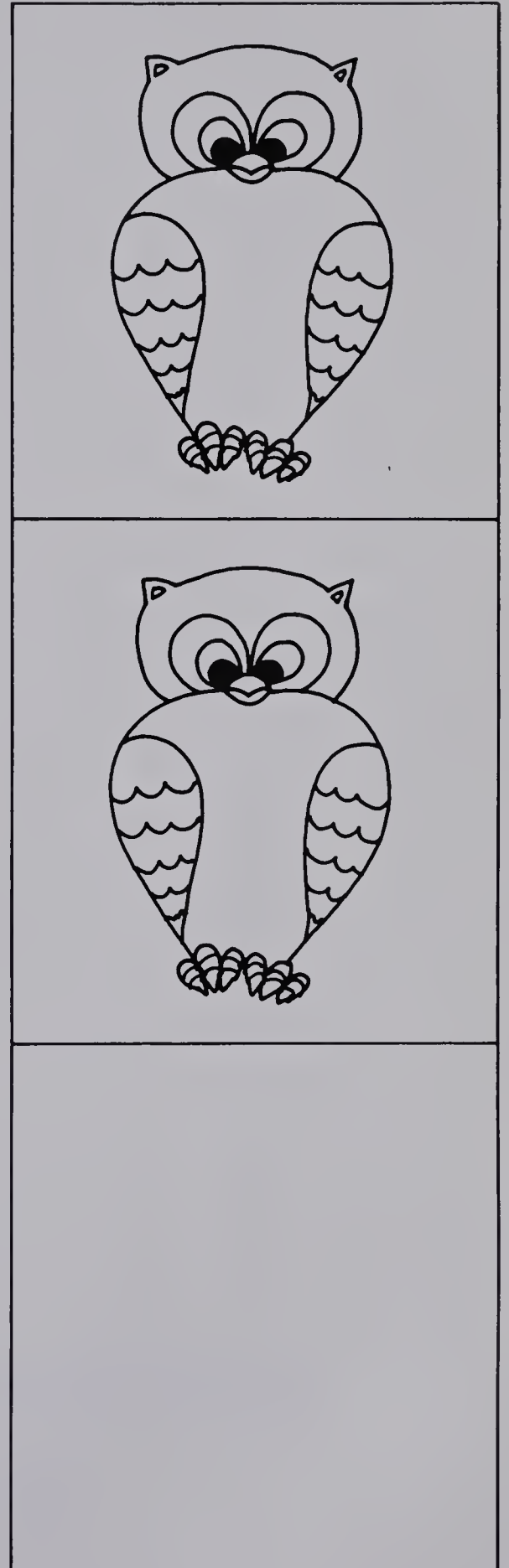
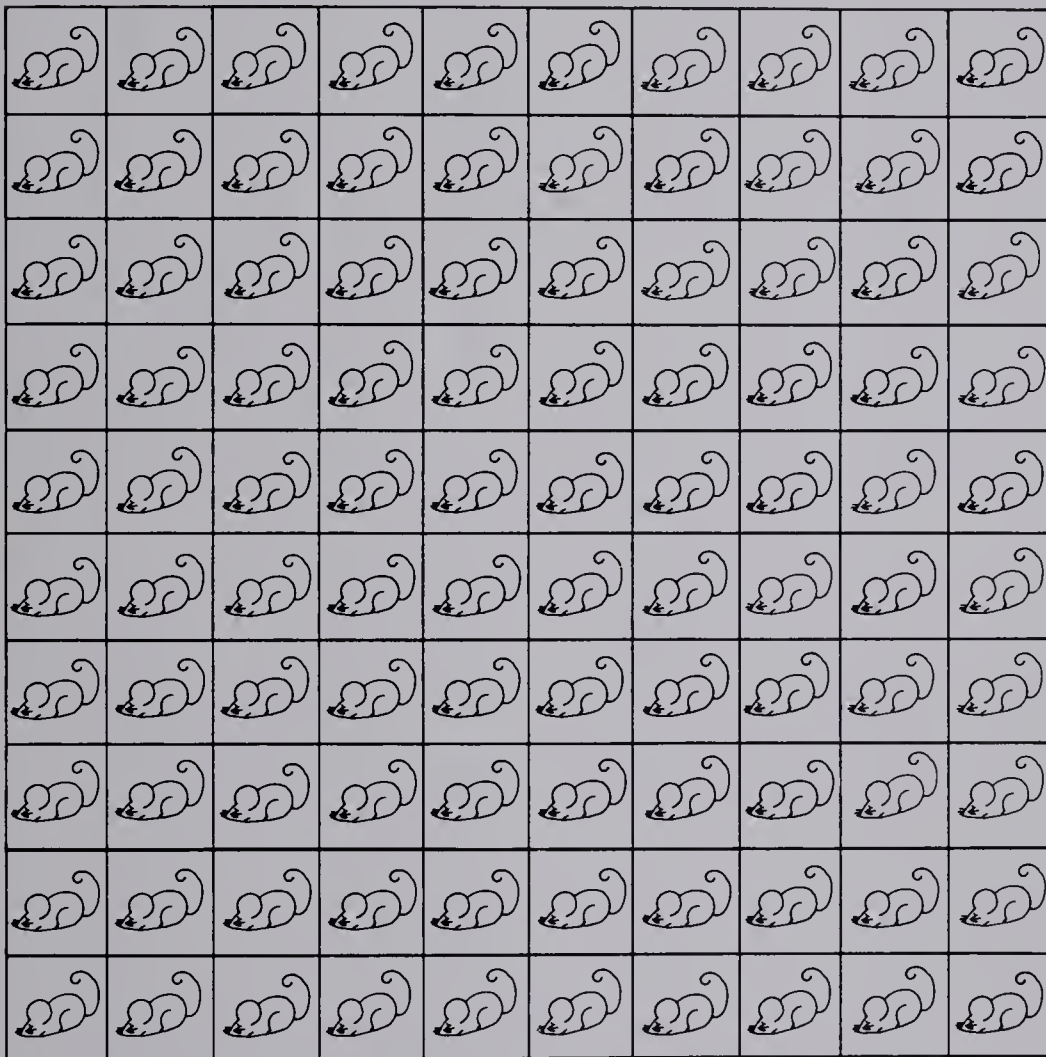
Remove and count the number of mice caught by each owl (at each drop). Keep all the mice from each owl catch in separate stacks. Record the data on the chart on page 311. You might want to have one team member make the catches while the other records the data.

For example, suppose generation 3 begins with 20 mice and 2 owls. You make a drop for the first owl and catch 7 mice. On the second drop, the second owl catches only 2 mice. The owls have caught a total of 9 mice. There are 11 mice left in Hoot Woods, and they reproduce 11 mice. The next generation will start with 22 mice. Because the first owl caught 7 mice, it reproduces 2 offspring for the next generation. The second owl caught only 2 mice; it starves and does not survive.

The data chart for this example would look like this:

<i>Generation</i>	<i>No. of Mice at Start</i>	<i>No. of Owls at Start</i>	<i>No. of Mice Caught</i>	<i>No. of Owls Starved</i>	<i>No. of Surviving Mice + Offspring</i>	<i>No. of Surviving Owls + Offspring</i>
3	20	2	9	1	11 + 11 = 22	1 + 2 = 3
4	22	3				

On this page are 100 mice and the owls.
See the last pages of the manual for the
other 300 mice.



Name _____ Date _____

<i>Generation</i>	<i>No. of Mice at Start*</i>	<i>No. of Owls at Start**</i>	<i>No. of Mice Caught</i>	<i>No. of Owls Starved</i>	<i>No. of Surviving Mice + Offspring</i>	<i>No. of Surviving Owls + Offspring</i>
1	3	1				
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						

*There always must be at least three mice at the start; if necessary, have mice migrate in.

**There always must be at least one owl at the start; if necessary, have one owl migrate in.

Now, gather the data for 25 generations. Remember to remove the caught mice and starved owls, and to add the offspring mice and owls. Always place mice randomly in the woods.

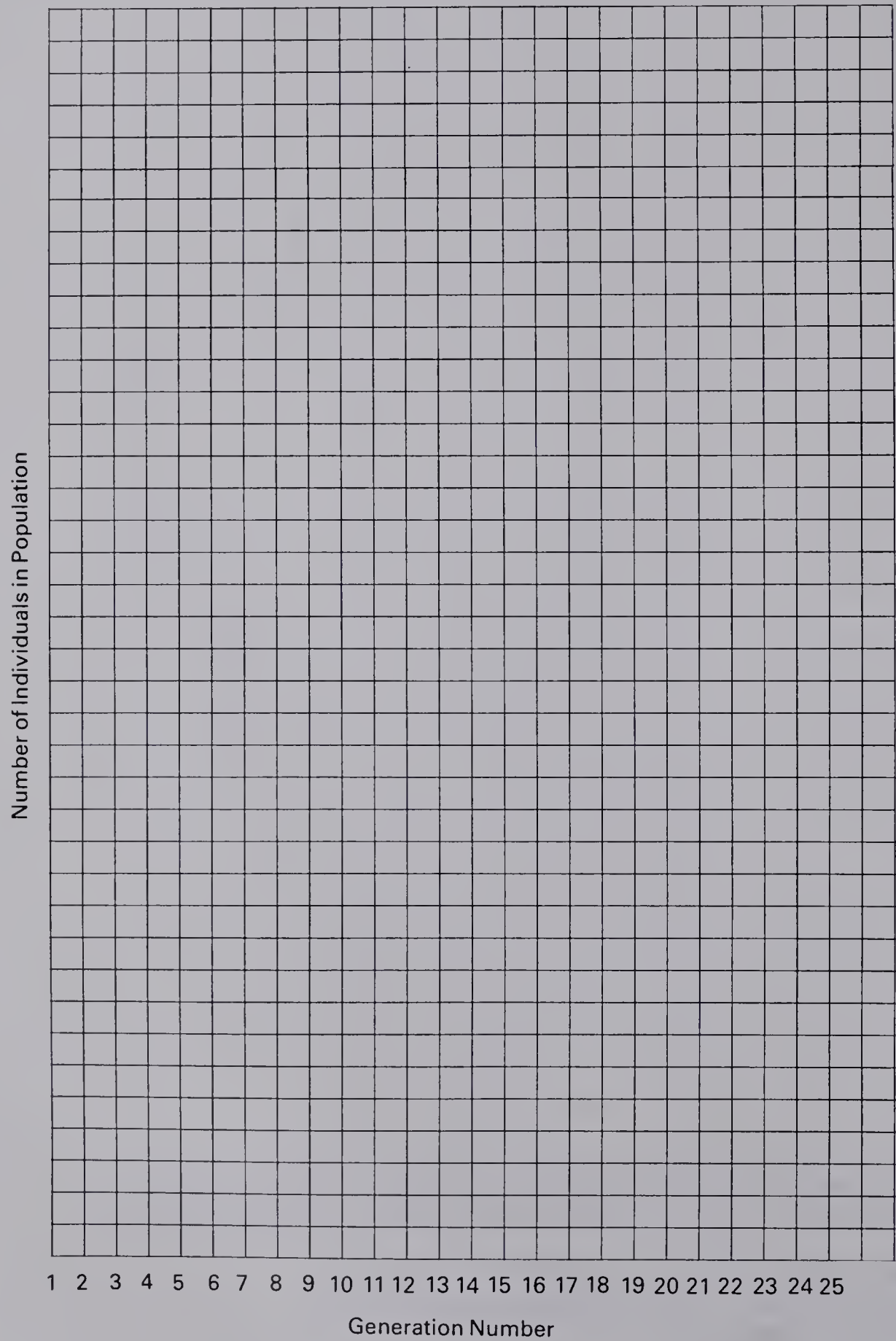
B. Graphing Data

When you have finished gathering data, plot your data on population size on the graph provided. Use Xs for the owl data and dots (•) for the

mouse data. Design the scale of the Y-axis so that most of the graph is used.

Connect the data points of each population. Your graph should have two distinct lines: one for the owl population and one for the mouse population.

Owl and Mouse Populations in Hoot Woods



ANALYSIS

1. Which population first increases in size?

2. Describe the pattern of the fluctuations in the sizes of the two populations.

3. By looking *only* at the graph, can you tell which species is the prey and which species is the predator? How can you tell?

4. Which species attains the greater number of individuals? Why?

5. What do you think would happen to the mouse population in Hoot Woods if the owls were all hunted to extinction? Why?

58 Population Growth

PURPOSE

To learn how populations grow.

MATERIALS

paper pencil

INTRODUCTION

Many species produce large numbers of offspring, which is necessary to ensure their survival. Think of all the seeds produced by a single dandelion flower. Very few actually survive and reproduce.

Vertebrates produce fewer offspring than do invertebrates, but a greater percentage of vertebrate offspring survive to maturity. This is certainly true of humans. In this lab you will investigate where the human population may be headed during your lifetime.

PROCEDURE

Your first population growth study will be of pennies.

Assume that you start with one penny. By cleverly investing, you double your money each day for twenty days. A progression of numbers in which each number is twice the preceding number is known as a geometric progression.

In your investment, the rate of increase is 100 percent per day. However, although the *rate* of increase is a constant, the actual *amount* of the increase varies.

1. On the data chart, calculate how much money you would have at the end of twenty days.

Double Your Money

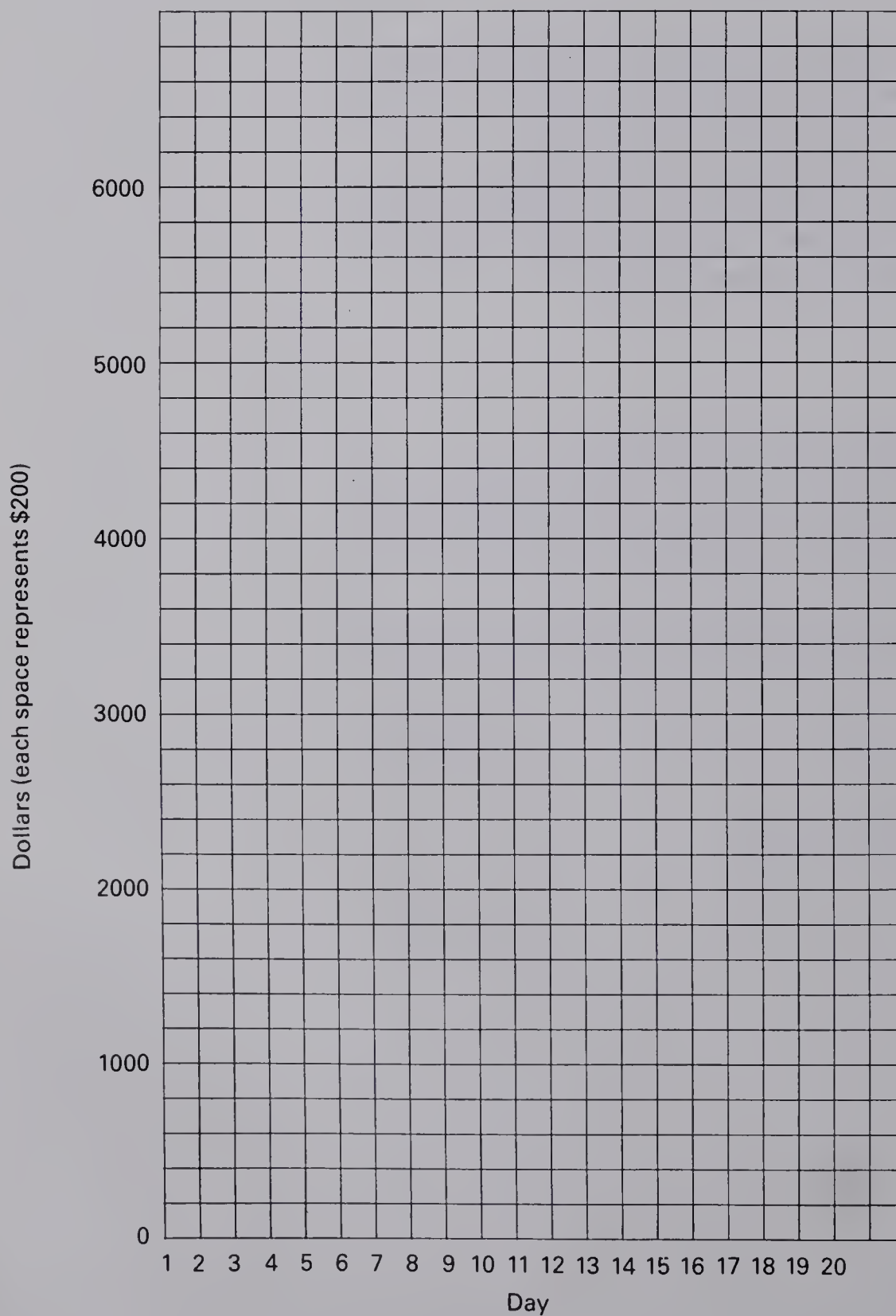
<i>Day</i>	<i>Amount</i>	<i>Day</i>	<i>Amount</i>
1	\$.01	6	
2		7	
3		8	
4		9	
5		10	

Double Your Money (continued)

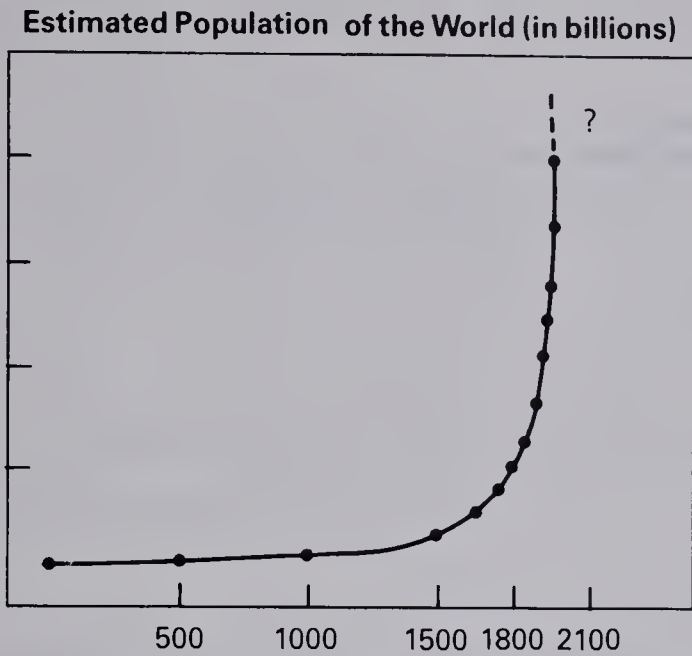
<i>Day</i>	<i>Amount</i>	<i>Day</i>	<i>Amount</i>
11		16	
12		17	
13		18	
14		19	
15		20	

2. Plot the data on the following graph. Note that each space on the Y-axis represents \$200.

Double Your Money
A Geometric Progression



Compare your graph with the graph of human population growth. The world population is increasing at a rate of approximately 1.7 per-cent per year.



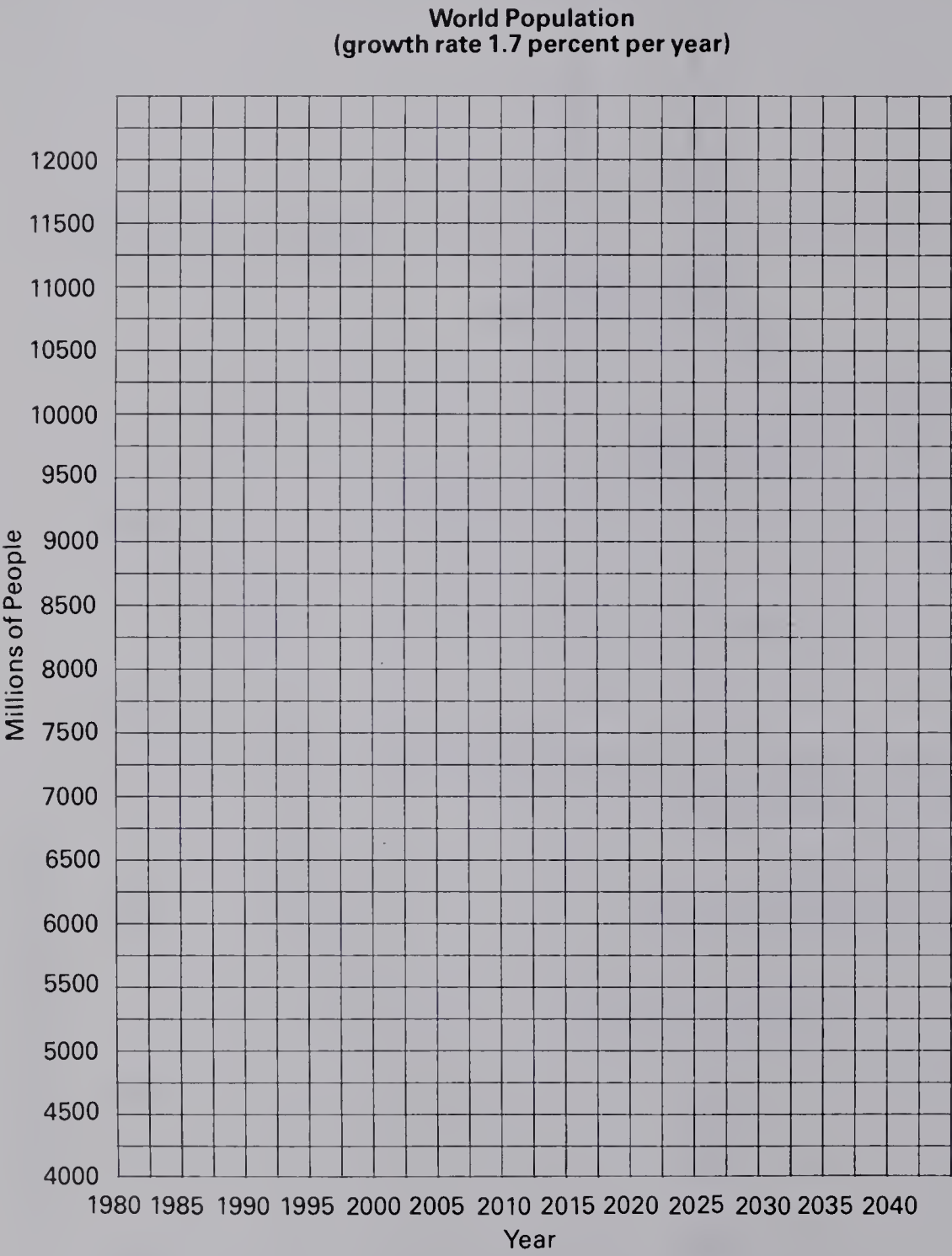
3. Since the rate of increase remains the same, how do you account for the sharp upward curve?

In the following chart, the world population growth is calculated to the year 2040, using 1.7 percent as a yearly increase.

World Population
(Growth rate 1.7 percent per year)

Year	Population in Millions
1980	4350
1985	4750
1990	5150
1995	5600
2000	6100
2005	6650
2010	7250
2015	7900
2020	8600
2025	9300
2030	10 150
2035	11 050
2040	12 000

4. Plot the data on world population growth on the following graph.



In 1980 the population of the United States approached 250 million people. Each space on the Y-axis of the world population graph represents an increase of 250 million people—the approximate population of the United States in 1980.

5. Starting from 1985 on the graph, determine the approximate number of years it will take the world population to increase by 250 million (assuming a steady growth rate).

6. Starting at 2035 on the graph, determine the approximate number of years it will take the world population to increase by 250 million (assuming a steady growth rate).

The total landmass of the earth is about 148 million square kilometres, only about half of which is inhabitable by humans. In 1980 the density of people in the world was about 29 people per square kilometre.

7. If the world population in the year 2040 were to reach 12 billion as shown on your graph, what would be the population density per square kilometre?

The limits of population growth are determined by the amount of space and energy (food) available. At a certain point, insufficient food or a change in behavior slow population growth.

Many species of vertebrates seem to have some instinctive method of limiting population size. Elephants mate less frequently if the herd becomes overpopulated. Rats kept in a cage and given adequate food and water will at first increase the size of their population. As the population increases, the young rats are neglected and only about 4 percent survive. Eventually, the rat population will stabilize.

ANALYSIS

8. Do you think that the earth's population will ever reach 12 billion? Why or why not?

9. In any population certain environmental factors tend to keep the population size under control. What environmental factors could control the size of the human population?

FOLLOW-UP

Write a paragraph predicting how the human population will ultimately stabilize and how that level could be maintained.

59 Adaptation in Leaves

PURPOSE

To compare the stomates of leaves of three plants from different biomes.

MATERIALS

prepared slides of leaf cross sections:

pondweed (*Potamogeton*)

lily

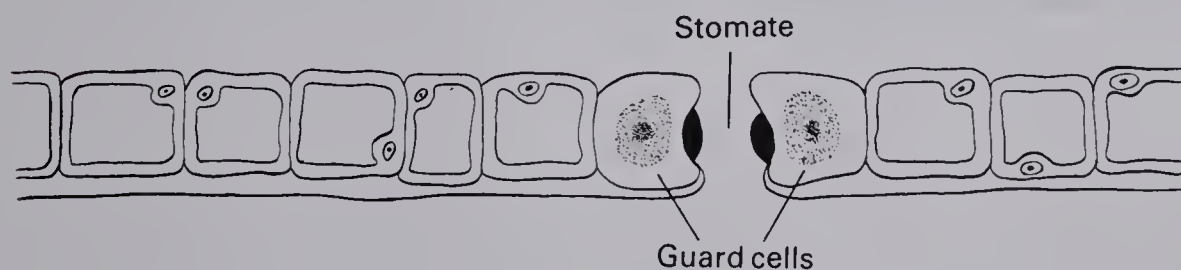
yucca

compound microscope

INTRODUCTION

There are many environments on the earth, and they can be as different as an ocean is from a desert. In each environment live organisms that are adapted to the climate, soil, water, food, and other conditions of that environment. In plants, for example, the amount of transpiration from leaves that occurs varies, depending on the species and the environment.

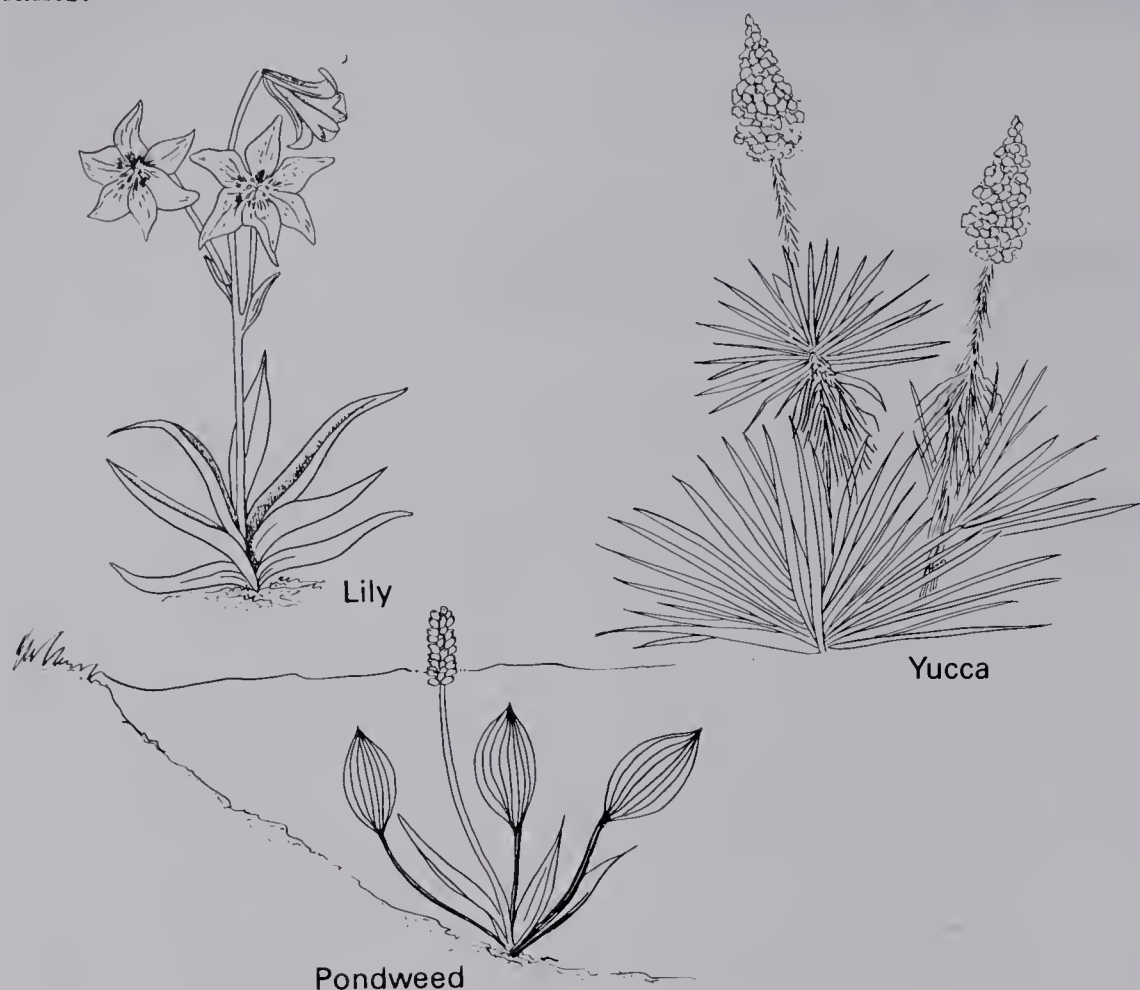
Transpiration occurs through stomates on the surfaces of leaves. Stomates also allow gas exchange between leaves and air, which is necessary for photosynthesis and cellular respiration. The position and number of stomates are vital to a plant's survival.



In this lab you will observe the leaves of plants that are adapted to three different habitats: hydric (wet), mesic (moderately moist), and xeric (dry). Hydrophytes grow in extremely wet areas or in water; their leaves may be submerged or flat on the water. Mesophytes grow in places with a moderate water supply. Xerophytes grow in deserts, where the climate is hot and water is scarce.

PROCEDURE

The leaves you will observe come from the lily, pondweed, and yucca plants.



Observe the slide of a lily leaf cross section under the compound microscope. Locate the stomates in the leaf epidermis. Note whether the stomates occur in both the upper and lower epidermis and estimate the number of stomates visible in the field of view under low power.

1. How many stomates are in the upper epidermis? the lower epidermis?

2. Draw a portion of the lily leaf showing the stomates.

Name _____ Date _____

Observe the slide of the pondweed leaf. Locate the stomates.

3. How many stomates are in the upper epidermis? the lower epidermis?

4. Draw a portion of the pondweed leaf showing the stomates.

Observe the slide of the yucca leaf. Locate the stomates, which may be a bit hard to find.

5. How many stomates are in the upper epidermis? the lower epidermis?

6. Draw a portion of the yucca leaf showing the stomates.

ANALYSIS

7. Which leaf is from a xeric habitat? How can you tell?

8. Which leaf is from a hydric habitat? How can you tell?

9. Which leaf is from a mesic habitat? How can you tell?

10. What other adaptations would you expect to find in the leaves of plants living in a xeric, mesic, or hydric habitat?

60 Sampling a Plant Community

PURPOSE

To learn the point-quarter technique for determining the plant density of an area.

MATERIALS

Per team of 2-4:

compass	scissors
masking tape	stake
metre stick	string

INTRODUCTION

Reading the landscape can deepen your understanding of biology. The trees of a wooded area have a story to tell. They are good indicators of the state of development of the area and the kinds of animals that live there.

In this lab you will learn the point-quarter technique of estimating the number of trees in a community. This simple technique can be used to sample any community of randomly distributed trees or large shrubs.

PROCEDURE

Read the following instructions carefully before collecting your data.

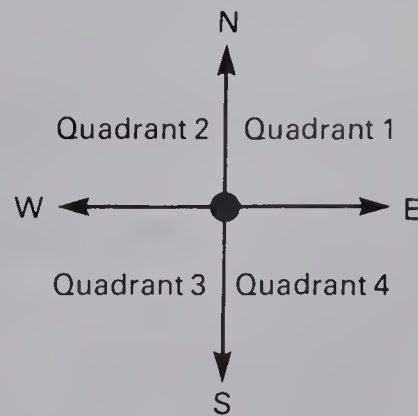
To study a stand of trees, first determine the size of the study area. Measure the perimeter, then calculate the area in square metres.

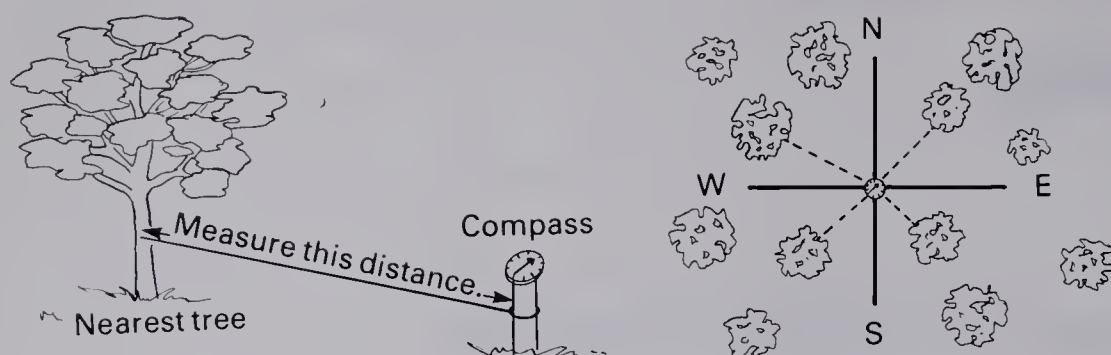
Next, determine the dominant kind of tree (such as oak, maple, or aspen) and learn how to identify it. If several kinds of trees are abundant, choose one for this study.

Each team will randomly select a point in the study area. Mark the point with a stake. Place the compass on top of the stake. Set up four 90° quadrants around the stake as illustrated. It is not necessary to mark off the quadrants. However, it is important to distinguish one quadrant from another.

Next, measure the distance from the stake to the nearest tree in each quadrant. Tie one end of the string to the stake and pull the string to the middle—not the edge—of the tree trunk. Measure the string distance with the metre stick.

Record the distance in metres on the data chart. Note whether the tree was dominant (the kind being studied) or another kind. Under "tree," write "dom." or "other."





Select 19 more points at random in the study area and repeat the procedure for each point.

Procedure Summary:

1. Select your first position.
2. Put stake in ground.
3. Determine quadrants by placing compass on stake.
4. Measure distance from stake to center of nearest tree in the first quadrant with string and metre stick.
5. Identify tree as "dominant" or "other."
6. Record data on chart.
7. Repeat steps 4-6 for each quadrant.
8. Remove stake and move to your next point.

After you have completed your measurements, make the following calculations. Add the four distances for each stake position and record the total in the "Total Distance" box. Calculate the grand total by finding the sum of the figures in the last column; record the grand total.

ANALYSIS

1. Calculate the mean distance between the trees and the stake.

$$\text{mean distance} = \frac{\text{grand total}}{80 \text{ (total number of measurements)}}$$

2. Calculate the mean area per tree by squaring the mean distance.

$$\text{mean area per tree} = (\text{mean distance})^2$$

Name _____ Date _____

Stake Position	Quadrant 1		Quadrant 2		Quadrant 3		Quadrant 4		Total Distance
	tree	distance	tree	distance	tree	distance	tree	distance	
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									

Tree being studied _____ Grand Total _____

3. Calculate the total density of all the trees in the area: divide the total study area by the mean area per tree.

$$\text{total density of trees in area} = \frac{\text{total study area (m}^2\text{)}}{\text{mean area per tree (m}^2\text{)}}$$

4. Calculate the relative density of the dominant tree. This will give the percentage of dominant trees in the study area. Also record the name of the dominant tree.

$$\text{relative density} = \frac{\text{total number of dominant trees on chart}}{\text{total number of trees examined (80)}} \times 100$$

On the chalkboard, record the team data for the total density of trees per area and the relative density for your tree.

5. Calculate the class average for total density and the class average for your tree.

6. How do your data compare with the class average?

7. What does the density and type of dominant tree in your study area tell you about the area? What types of animals would live there? Is this a community in transition (for example, from marsh to forest), or an established climax community?

61 Movement in Plants

PURPOSE

To learn how plants respond to certain stimuli.

MATERIALS

12 oat seedlings

4 plastic foam cups

vermiculite

unidirectional light source

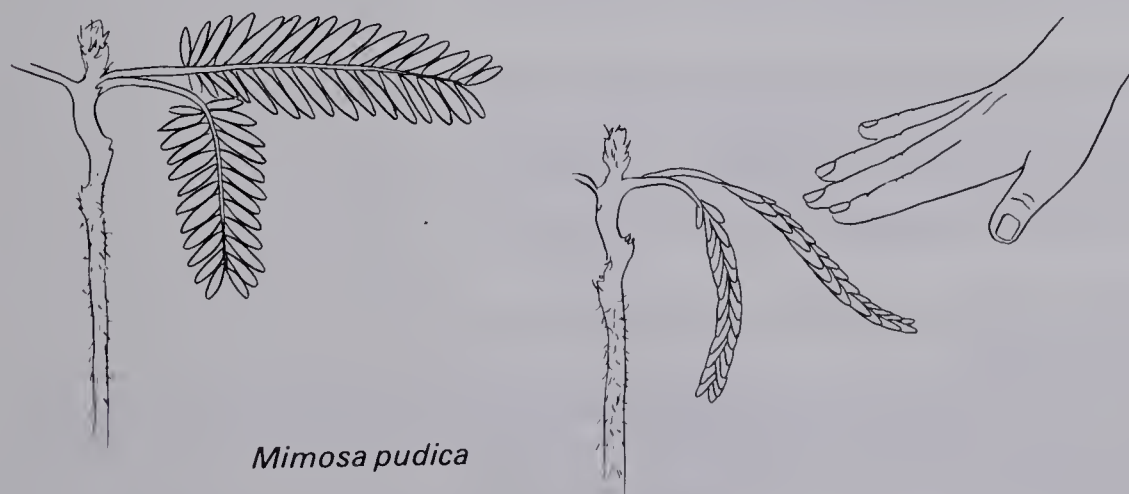
aluminum foil

INTRODUCTION

Plants, like animals, respond to stimuli in their environment. Stimuli such as light, gravity, touch, and water can cause movements in plants.

There are two main types of plant movements. Movements toward or away from a stimulus are called tropic movements, or tropisms. Plant movements in response to a stimulus, but in a direction independent of the stimulus, are called nastic movements.

You may have seen two common nastic movements. When the leaves of *Mimosa pudica* are touched, the leaves droop. When insects walk on the leaves of the carnivorous Venus' flytrap, the leaves fold up and trap the insects. No matter where the *Mimosa* leaves are touched or in which direction the insects walk on the flytrap, the plant movements do not vary.



Tropic movements, on the other hand, are either positive or negative. If the plant moves toward the stimulus, the movement is positive. If it moves away from the stimulus, the movement is negative.

Movements are classified according to the stimulus. If the stimulus is light, the response is called phototropism or photonasty. Other movements are geotropism and geonasty, a response to gravity, and

hydrotropism and hydronasty, a response to water. If you know that *thigmo* means "touch," you can guess what a thigmotropism or thigmonasty is. For example, when a vine touches a fence, then wraps itself around the fence, it is exhibiting a positive thigmotropic response.

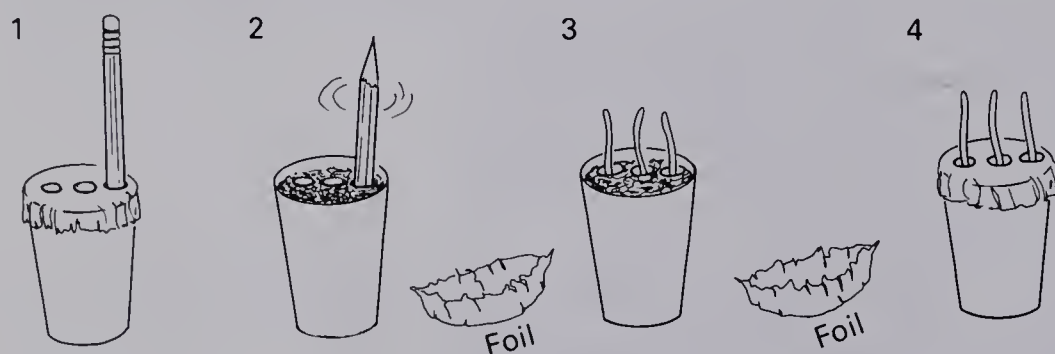
In this lab, you will perform two experiments to study the effect of light and of gravity on plant growth.

PROCEDURE

A. Gravity

Fill two plastic foam cups to the top with vermiculite or another potting medium. Wet the vermiculite thoroughly. If necessary, add more vermiculite to completely fill the cups. Cover each cup with your fingers and drain all excess water into the sink, taking care not to let any vermiculite fall out.

Cover the top of each cup with aluminum foil. With the sharp end of a pencil, poke three holes in a row through the middle of the foil. Push the pencil down into the vermiculite to mark the position of the holes. Remove the foil. With the blunt pencil end, enlarge each marked hole in the vermiculite to a depth of about 1 cm.



Obtain six oat seedlings and plant one in each hole. Pat the vermiculite around the seedlings so that they will remain in place. Replace the foil over each cup, carefully threading the seedlings through the holes. If necessary, enlarge the holes in the foil so that it does not touch the seedlings.

Label the cups *A* and *B* and write your name on them. Put the cups in a spot that receives uniform room light, as designated by your teacher. This is to ensure that light will not be a variable in the experiment—both plants will receive the same amount of light from the same direction.

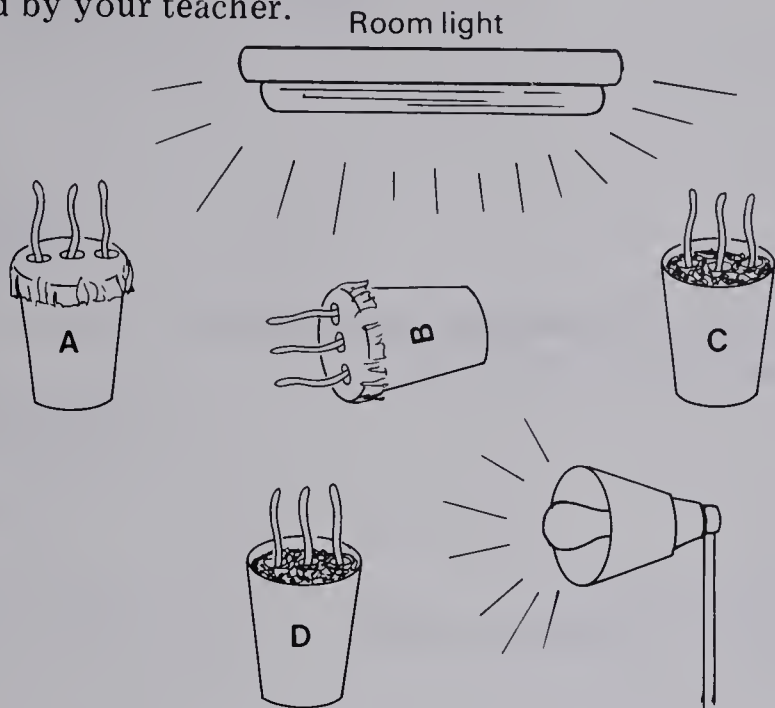
Place cup *A*, the control, upright. Place cup *B*, the experiment, on its side (the foil will hold in the vermiculite).

The cups will remain in place for one to two days. When the seedlings' growth pattern is clearly established, you will collect data (in part C). Meanwhile, after you have set up this experiment, proceed to part B.

B. Light

Prepare two cups, each containing three seedlings, as you did in part A—but do not cover them with foil. Label the cups *C* and *D* and write your name on them.

Place cup *C* upright with cups *A* and *B* in the evenly lighted spot. Place cup *D* upright in a spot with a unidirectional light source, as designated by your teacher.



The cups will remain in place for one to two days. When the seedlings' growth pattern is clearly established, you will collect data (in part C).

C. Data Collection

Examine the seedlings in the four cups. Note the pattern of growth.

1. Fill in the chart with data collected from your experiments. Indicate whether the movement is a phototropism, photonasty, geotropism, geonasty, positive, negative, or none of these.

<i>Cup</i>	<i>Kind of Movement</i>
<i>A</i>	
<i>B</i>	
<i>C</i>	
<i>D</i>	

Remove the seedlings from cup *A* and gently shake loose any vermiculite on the roots. Examine the growth pattern of the roots.

2. In what direction did the roots grow?

3. Predict the direction of root growth in cup *B*.

Remove the seedlings from cup *B* and examine the growth pattern of the roots.

4. Was your prediction confirmed? In what direction did they grow? What is this growth response called?

ANALYSIS

5. In part A, foil was put on cup *B* to hold in the vermiculite when the cup was placed on its side. Why was foil also put on cup *A*?

6. Cup *A* was the control for cup *B*. Could cup *A* also have served as the control for cup *D*? Why or why not?

7. The petals of certain flowers open in the presence of bright light and close in the dark. Are these movements tropic or nastic? Why?

8. How could a positive phototropism benefit a plant?

9. How could the negative geotropism you observed benefit the plant? How could the positive geotropism you observed benefit the plant?

62 Protist and Animal Behavior

PURPOSE

To learn how several organisms respond to stimuli.

MATERIALS

Per team of 2:

brine shrimp culture	dissecting microscope
6 isopods	eyedropper
<i>Paramecium</i> culture	hand lens
acetic acid or 0.1 M hydrochloric acid	large beaker
aluminum foil	ring stand
cardboard shoebox	2 rubber stoppers
compound microscope	2 test tubes
slides and coverslips	2 test tube clamps

INTRODUCTION

Animals exhibit two basic kinds of behavior: inherited and learned. Animals with complex nervous systems can learn new behaviors and adjust their inherited behaviors to new situations. In animals without complex nervous systems, almost all behavior is inherited. Inherited behaviors exhibit predictable responses to stimuli.

In this lab you will observe the inherited behaviors of three organisms. The behaviors can be classified as either kinesis or taxis. A kinesis is a random movement in response to a stimulus. A taxis is a movement toward a stimulus (positive taxis) or away from a stimulus (negative taxis).

Taxes and kinesis are described according to the type of stimulus involved. For example, a movement away from heat ("thermo-") is a negative thermotaxis. Other stimuli are light ("photo-"), gravity ("geo-"), touch ("thigmo-"), chemicals ("chemo-"), water ("hydro-"), moisture ("hygro-"), and electricity ("galvano-").

PROCEDURE

Work in teams of two. When necessary, one person can carry out procedures while the other makes observations.

A. Paramecia

Paramecia are among the many protists that live in pond water. Their behavior enables them to stay in the best habitat for survival, to feed on bacteria, and to avoid predators.

Response to Touch With an eyedropper, take a few drops from the cloudy part of a *Paramecium* culture. Place the drops on a clean slide and add a coverslip. Take turns observing the slide under low power with the compound microscope.

1. What happens when the paramecia bump into each other?

2. What is this behavior called?

Response to Chemicals Have one team member put a drop of acetic acid at the edge of the coverslip while the other member watches the paramecia through the microscope.

3. What do the paramecia do?

4. What is this response called?

Response to Gravity Transfer 5 mL of *Paramecium* culture to a test tube and add enough water to fill the tube. Insert a stopper and attach the tube to a ring stand with a clamp. Repeat the procedure for another test tube, but attach it in an upside-down position. Adjust the clamps so the test tubes are at the same height. The setup should receive general room light.

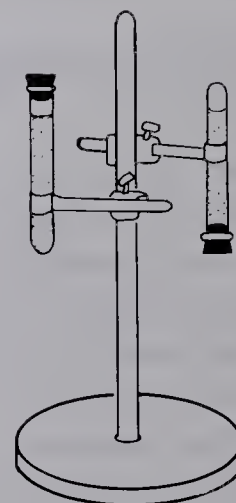
Examine the test tubes with a hand lens.

5. How do the paramecia respond to gravity?

6. What is this behavior called?

B. Brine Shrimp

Brine shrimp live in saltwater lakes. They are often used as food for aquarium fish.



Response to Light Put about 5 mL of the brine shrimp in a large beaker. Completely cover the top and sides of the beaker with aluminum foil. With a pencil, poke a hole through the foil on top, near the edge. Leave the beaker for about ten minutes, then remove the foil and watch the animals.



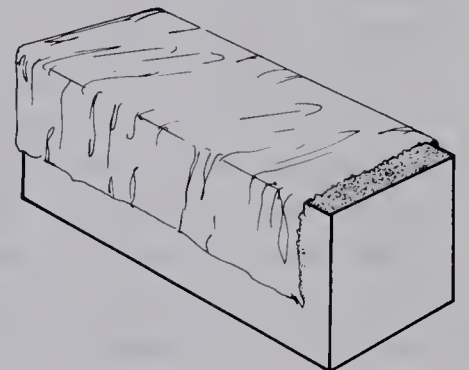
7. Where are the brine shrimp?

8. What is this response called?

C. Isopods

If you turn over a rock or a log, you are likely to find some isopods (also called pill bugs or sow bugs) underneath. Unlike their aquatic crustacean relatives, shrimps and crayfish, isopods live on land. However, they require a moist habitat.

Response to Light Put one layer of paper towels, evenly moistened, in the bottom of a shoebox. Add six isopods. Cut off a 3-cm strip from one end of the shoebox top. Cover the shoebox with the cut top. Leave the setup for five minutes, then remove the top and note the position of the isopods.



9. Where are most of the isopods?

10. What is this response called?

Remove the isopods and paper towels from the shoebox.

Response to Moisture Put a fresh layer of paper towels in the bottom of the shoebox. Moisten one end of the towel, and add six isopods. Observe the isopods for five minutes.

11. Do they move in a specific direction or randomly?

12. Where do they congregate?

13. What is this response called?

ANALYSIS

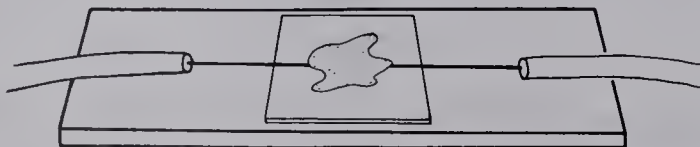
14. Which of the experimental organisms would you expect to have the smallest capacity for learning, and to have the most predictable behaviors? Why?

15. Suppose you carried out the experiment on response to light, substituting green euglenas for paramecia. What result would you expect? Why?

FOLLOW-UP

Design additional experiments to test the organisms' behavioral responses. For example, you can test the response of paramecia to electricity (galvanotaxis).

Put 5 mL of the *Paramecium* culture on a microscope slide and add a coverslip. Remove the insulation at the ends of three wires. Connect the end of one wire to the positive pole of a dry cell and the end of another to the negative pole of a second dry cell. Connect the two cells to each other in series with the short wire. Holding the insulated part of the long wires, insert one wire under each side of the coverslip. To which pole are the paramecia attracted?



63 Antibiotics and Bacteria

PURPOSE

To determine the effect of certain antibiotics on bacteria.

MATERIALS

broth cultures of <i>Bacillus subtilis</i> and <i>Escherichia coli</i>	Bunsen burner
	forceps
antibiotic disks:	incubator
penicillin, aureomycin, and streptomycin	spreading rod
2 sterile 9-cm petri dishes containing nutrient agar	2 sterile dropping pipettes
	wax pencil
ethyl alcohol	

INTRODUCTION

An antibiotic is a substance that is harmful to living organisms (*anti* means “against,” and *bio* means “life”). Most antibiotics are harmful to specific organisms. If an antibiotic were toxic to all organisms, it would have little value as a cure for a disease.

In the microenvironment of a petri dish, it is possible to determine how effective an antibiotic is for controlling specific types of bacteria. A small paper disk impregnated with antibiotic is placed in a dish containing bacteria. If the antibiotic is effective, the bacteria will not survive in the area around the disk. The larger the area, the more effective the antibiotic.

PROCEDURE

Obtain two sterile petri dishes containing nutrient agar. With a wax pencil, write the letter *B* on the bottom of one dish and *E* on the bottom of the other.

With a sterile dropping pipette, transfer two drops of the *Bacillus subtilis* broth culture to dish *B*. Put the used dropper into a beaker of alcohol or another place designated by your teacher. The dropper must be resterilized before it is used again.

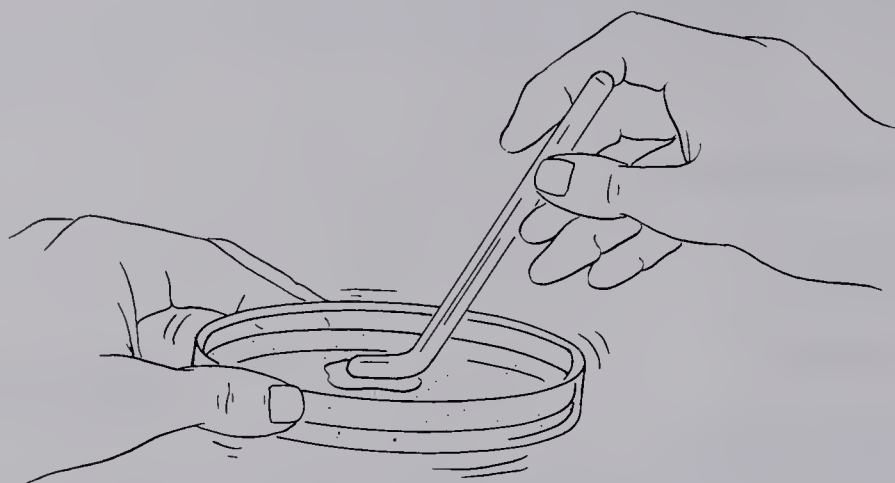
With a second sterile pipette, transfer two drops of *Escherichia coli* broth culture to dish *E*.

Take Care: To avoid contaminating the cultures, use sterile instruments when working with each culture. When sterilizing the instruments, do not heat them to red-hot incandescence—this will damage the instruments.

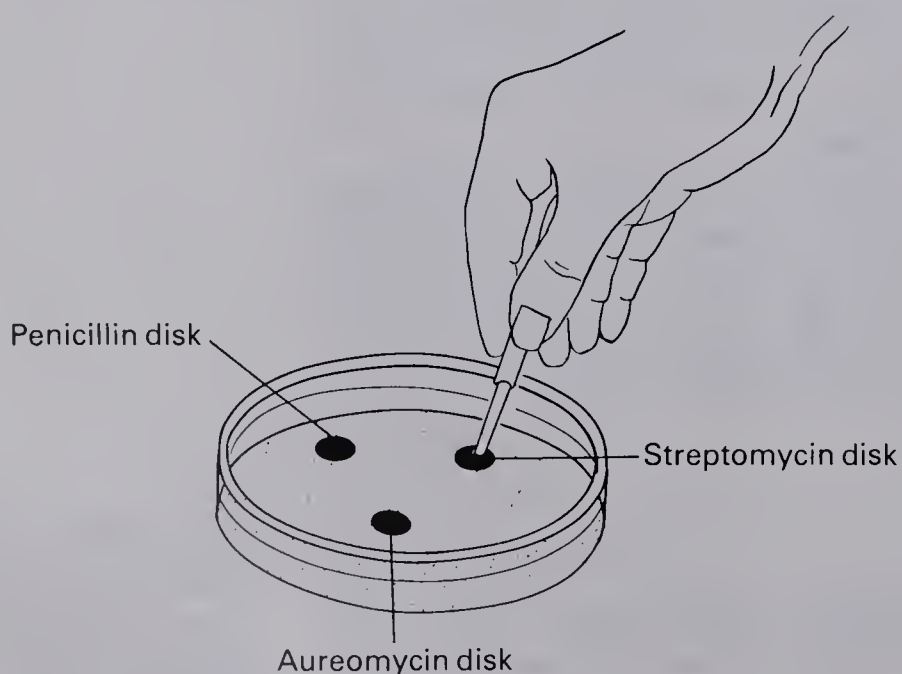
Obtain a spreading rod and sterilize it as follows. Dip the flat portion in alcohol, then remove the rod from the alcohol. Carefully ignite the alcohol on the rod over a Bunsen burner and let all of it burn off.

Place the flat end of the rod on the agar in dish *B* and rotate the petri dish to spread the drops of culture. Cover the whole dish with the culture, taking care not to break the surface of the agar. Resterilize the rod before you spread the culture in dish *E*.

Caution: Ignite the alcohol on the spreading rod only—do not get the container of alcohol near the open flame.



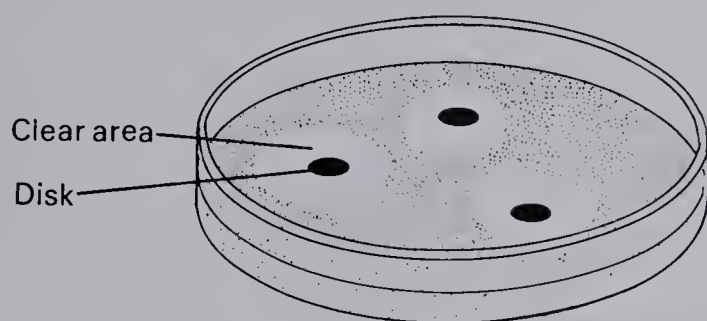
Place one of each type of the three antibiotic disks on the agar in each dish, as illustrated. If no dispenser is available, use sterile forceps to put the disks in place. Sterilize the forceps before doing the second dish, using the flaming alcohol technique. Cover both dishes.



Three minutes after placing the antibiotic disks on the agar, turn the dishes upside down. If the disks fall off the agar, reposition them with sterile forceps.

Incubate the dishes, in the upside-down position, in an incubator at 37°C. If no incubator is available, put the dishes in a dark place designated by your teacher.

Observe the dishes each day for several days. Clear areas around the disks are due to destruction of bacteria. When enough bacterial growth occurs to distinguish the clear areas, the experiment is complete.



1. Draw the two dishes showing the clear areas around the antibiotic disks. Label the dishes and disks.

ANALYSIS

2. Is the same antibiotic equally effective against both types of bacteria? How can you tell?

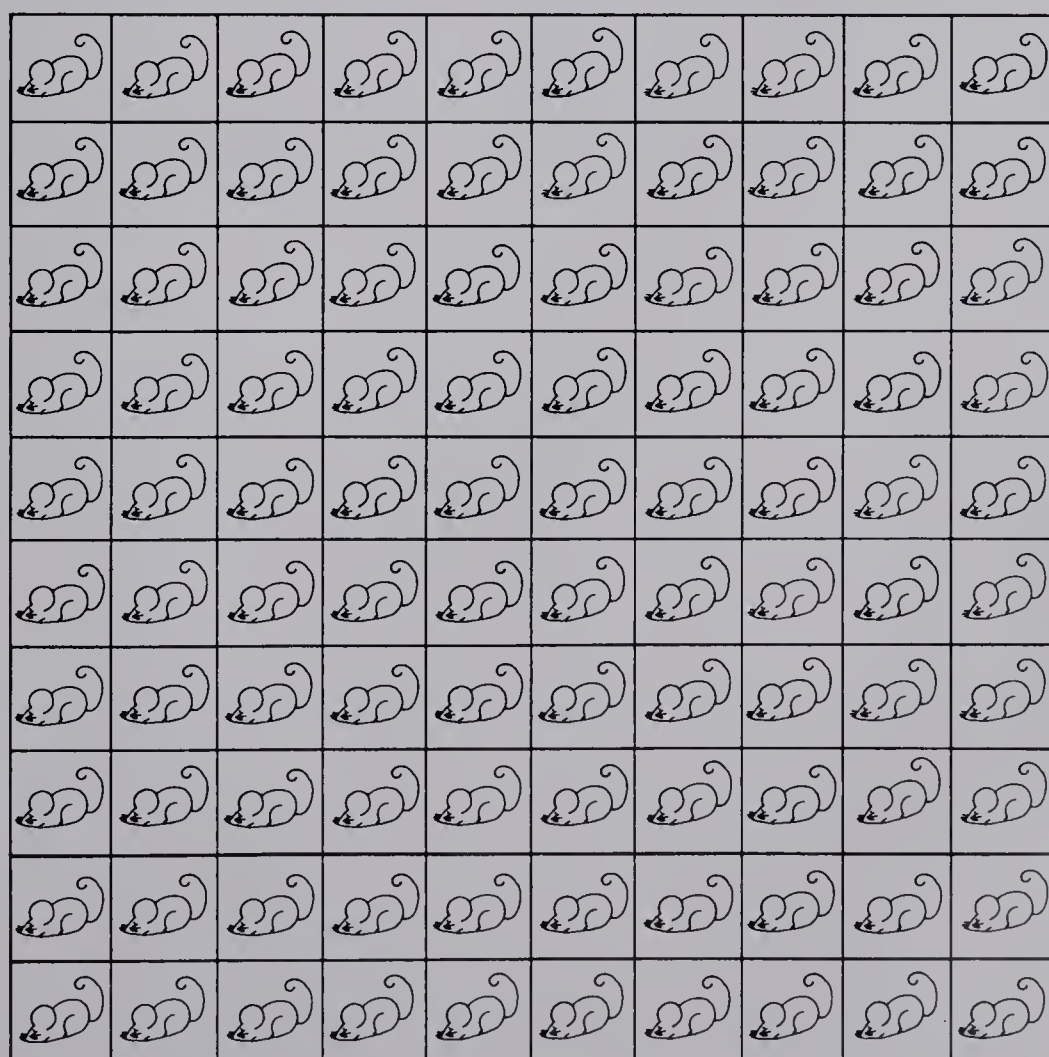
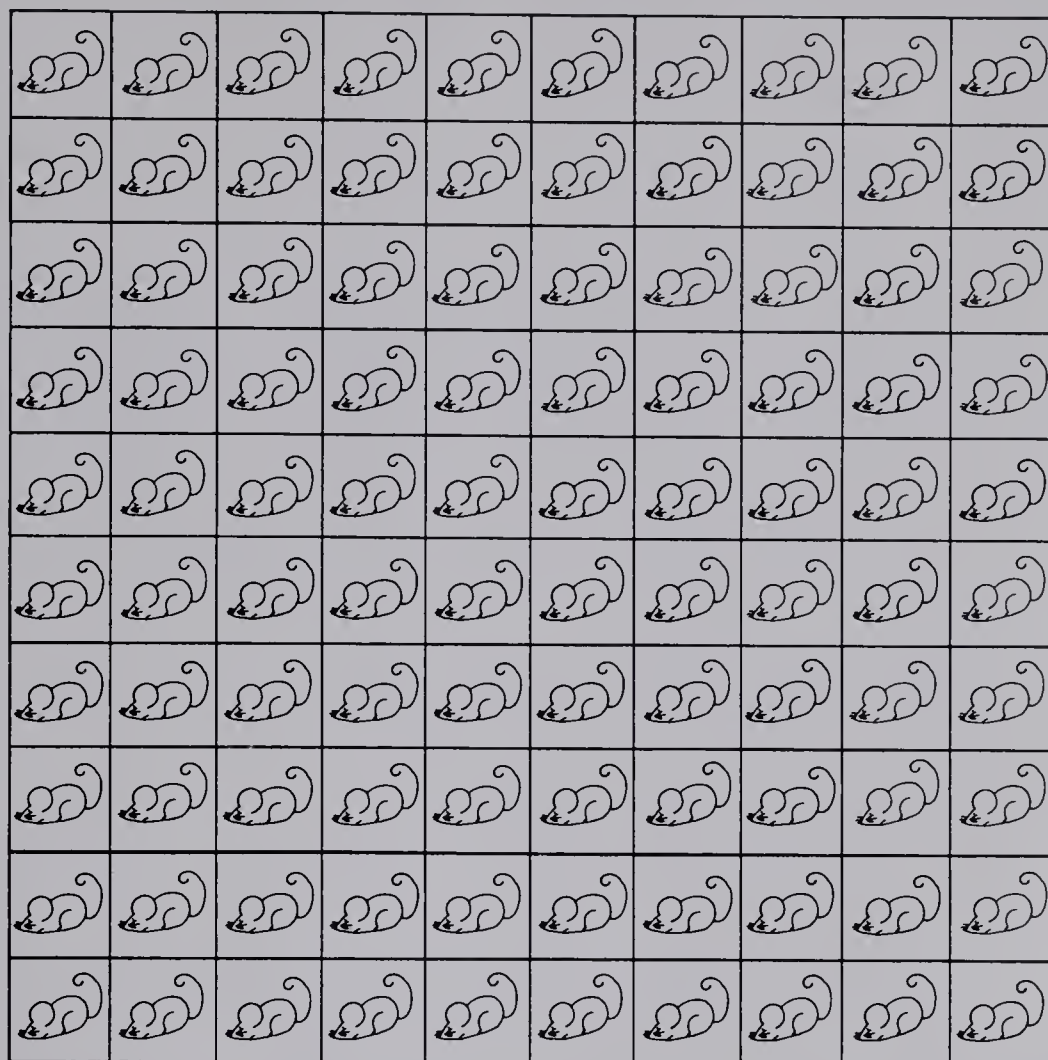
3. Which antibiotic works most effectively against *Bacillus subtilis*?

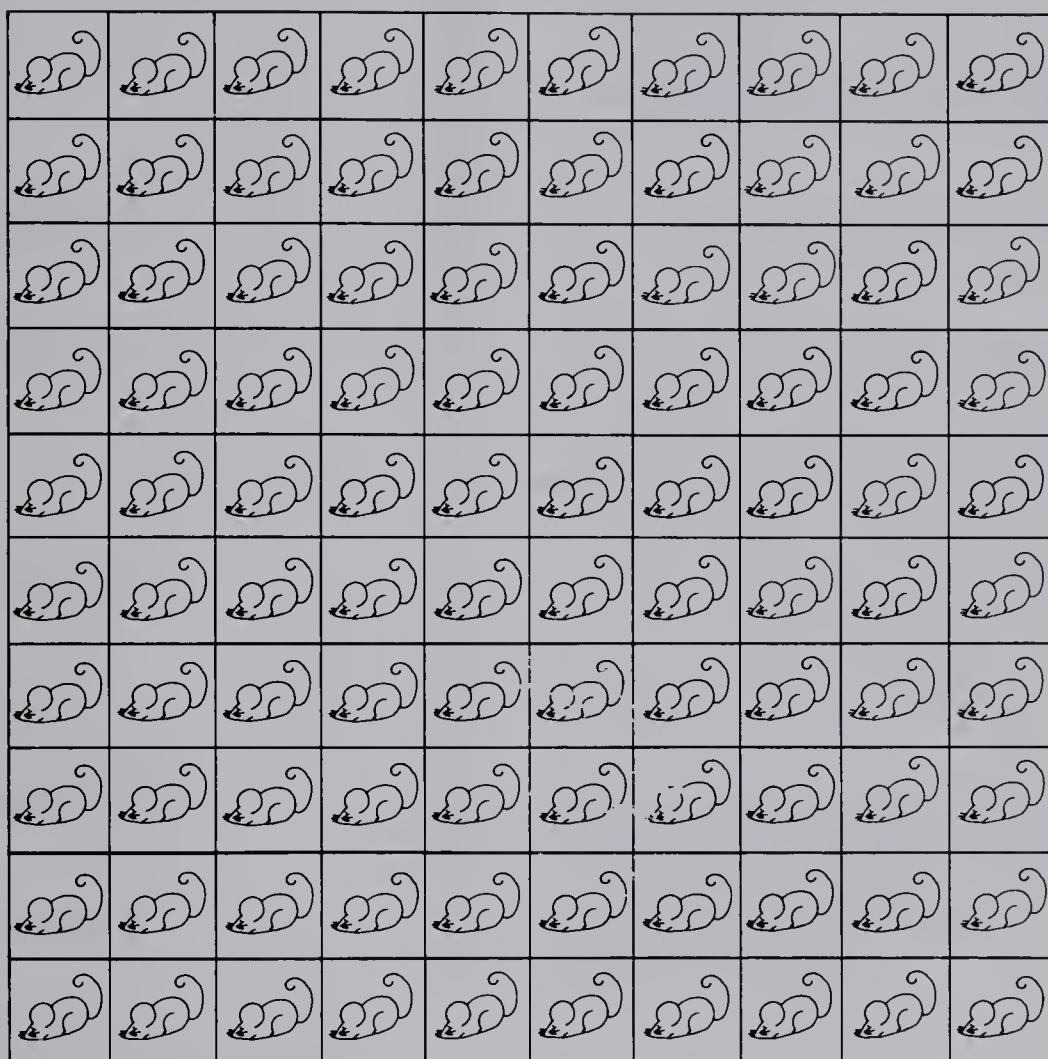
4. Which antibiotic works most effectively against *Escherichia coli*?

5. Why can one kind of bacteria survive in the presence of an antibiotic that is toxic to another kind of bacteria?

6. As organisms grow and reproduce, mutations sometimes occur in the nucleic acids of the cell nucleus. These mutant cells may have nutritional requirements different than the normal cells have. In your petri dishes, there might be a few small bacterial colonies in the clear areas surrounding the antibiotic disks. What would happen if you cultured these bacteria and then tested them with the same antibiotic? Why?

7. Why must medical researchers continually search for new antibiotics?





Safety Guidelines for the Biology Laboratory

The following guidelines are recommended for anyone who performs laboratory activities that involve handling potentially hazardous chemical, physical, and biological materials. Some of these are suggested safe procedures to use and others are rules that must be followed to avoid a hazardous situation.

Fumes	Use a hand to waft fumes or odors toward the nose rather than sniffing directly.
Heated Test Tubes	Never point the open end of a heated test tube toward anyone.
Glassware	Avoid handling broken or chipped glassware. Never force glass tubing into a rubber stopper.
Sharp Edges	Use care in handling dissecting tools, especially scalpels. Make sure the specimen being dissected is securely positioned on a flat surface. Gently position coverslips and avoid handling edges roughly.
Water and Acid Mixing	Do <i>not</i> pour water into acid—an explosion could result; carefully introduce acid into water in a steady stream while mixing slowly.
Burners and Flames	Never heat alcohol or other flammable liquids over an open flame. Keep long hair tied back when using burners and flames.
Poisons	Do <i>not</i> taste any solution or chemicals used in the laboratory (unless specifically instructed to do so). There are regulations forbidding consumption of food or drink in any laboratory.
Turn Off and Disconnect	Remember to turn off water faucets, hot plates, and burners and disconnect microscope cords and any electrical equipment after using or at the end of each laboratory session.
Dangling Cords	Fasten microscope cords so that they do not hang down where they could be tripped over.
Sunlight and Microscopes	Using sunlight to illuminate objects viewed through a microscope lens may cause permanent damage to the eye. Use artificial light from a lamp or indirect lighting.
Toxic and Flammable Substances	Handle with care. Follow the instructions of your teacher in dealing with specific substances.

First Aid in the Laboratory

If an accident occurs in the laboratory that results in injury, whether minor or serious, it should be *reported immediately* to your teacher or laboratory supervisor. For minor injuries certain first aid steps can be taken right away (these are listed below). Find out where the first aid kit is kept in the laboratory and learn how to use the materials contained in it to treat minor cuts and burns. Know the locations of first aid supplies such as baking soda and boric acid and have small quantities handy when working with acids and bases. Find out where the nearest phone, fire alarm, and fire extinguisher are located.

Keep in mind that no injuries are likely to occur in the laboratory if precautions are taken; all the injuries listed below can easily be prevented from happening. The following table lists the common types of injury that can occur in a laboratory and the appropriate first aid response for each.

Type of Injury	First Aid
Burns	Immediately flush with cold water until burning sensation is lessened.
Cuts, bruises	Follow instructions in first aid kit. Pressing directly on minor cut will stop bleeding in a few minutes. Apply cold compress to bruise to reduce swelling.
Fainting	Provide fresh air (open a window for instance) and have the person recline so that the head is lower than the rest of the body.
Eye injuries	Flush eye(s) immediately with plenty of water for several minutes. Use an eyewash fountain or bottle if available. If a foreign object lodges in the eye(s) do not allow the eye(s) to be rubbed.
Poisoning	Note what substance was responsible for the poisoning and alert the teacher immediately.
Spills on skin	Flush with water if substance is not a strong acid or base. Acid spills - Apply baking soda. Base spills - Apply boric acid.

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